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INBORN ERRORS OF METABOLISM

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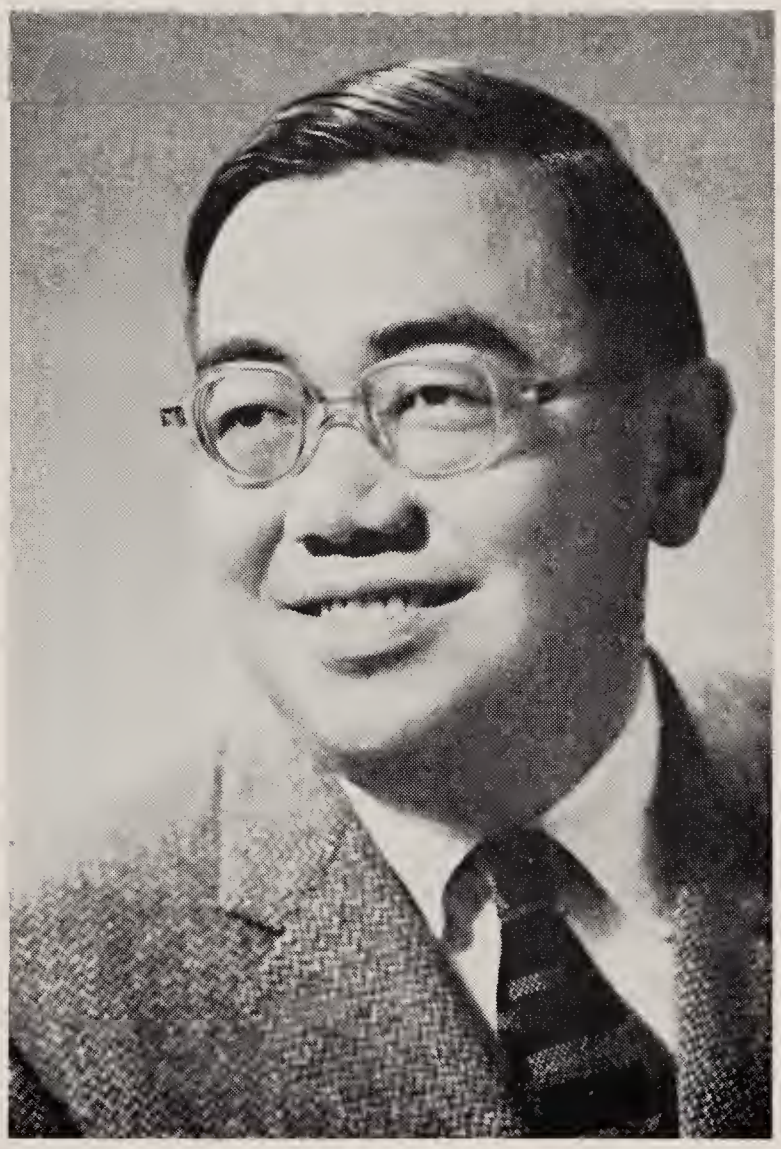
Guest Editorial



Dr. David Yi-Yung Hsia was born in Shanghai, China and received his medical training at Harvard Medical School. After completing his hospital work at the Charity Hospital in New Orleans, the Children's Hospital of Philadelphia, and the New York Hospital, he spent five years as a post-doctoral fellow in pediatrics and biochemistry at Harvard. Subsequently, he worked at the Galton Laboratory at University College in London in the field of human genetics. Since 1957, he has been on the faculty of Northwestern University Medical School. Dr. Hsia has just been appointed Professor and Chairman of the Department of Pediatrics at the Loyola University-Stritch Medical School in Chicago.

Dr. Hsia has worked actively on phenylketonuria, galactosemia, and various other hereditary metabolic diseases. His text on "Inborn Errors of Metabolism" has had two editions and has been republished in South America, Taiwan, and Japan. In 1965, Dr. Hsia was awarded the E. Mead Johnson Award for Research in Pediatrics by the American Academy of Pediatrics in recognition of his outstanding contributions in developing the field of human biochemical genetics.

In this guest editorial for an issue devoted to "Inborn Errors of Metabolism", I thought that it would be interesting to recount the several influences which brought about a reawakening of interest in this area during the mid-1950's.

Although today we all realize that it was Sir Archibald Garrod who opened up this field by delivering the Croonian Lectures before the Royal College of Physicians in 1908, it should be pointed out that his work and publications were pretty inaccessible; I remember spending several days digging through the stacks of both the Harvard Medical School Library and the Boston Medical Library looking for the original edition of "Inborn Errors of Metabolism" which was published in 1923. This search was unsuccessful and one had to settle for reading the original papers which were published in the *Lancet* in 1909.

Instead, one was much more heavily influenced by the contemporary research going on in human genetics and particularly human biochemical genetics. In 1949, Pauling had published on the difference in the isoelectric point of hemoglobin from patients with sickle cell anemia from normal subjects and he had proposed that these diseases should be called "molecular diseases". This represented considerable foresight since Ingram had not as yet shown the nature of the amino acid substitution in hemoglobin S. About the same time, both Neel from the United States and Beet from Africa showed that sickle cell anemia was transmitted by an autosomal recessive gene, where the heterozygous carrier with one normal and one abnormal gene would show certain hematological and subsequently certain electrophoretic differences. All of these

observations were very disturbing to "classical" geneticists who thought that traits were transmitted either as dominants or recessives as in drosophila.

At about this time, Stanbury and others started to make a series of observations on patients with congenital goiter and suggested that this group of conditions could be viewed in the same way as Garrod's "Inborn Errors of Metabolism" or Pauling's "molecular diseases". In the same way, some progress was made in our understanding of phenylketonuria and particularly with the use of a low-phenylalanine diet in the prevention of the mental retardation in that disorder. Again, it was felt that this was another condition which might be included in this category.

Perhaps the most systematic approach to these conditions was taken by Professor Harry Harris from London. In 1953, he published a soft cover monograph entitled *An Introduction to Human Biochemical Genetics*, where he pointed out that such diverse data on cystinuria, blood group substances, taste of phenylthiourea; and amino isobutyric acid excretion could be viewed in the same light in terms of genetics and biochemistry.

However, these concepts were not quickly accepted by the medical profession. The view was frequently expressed that these defects were merely medical curiosities, much like freaks in a circus. As late as 1956, a short manuscript unifying all of these concepts was rejected by one of the leading medical journals as being somewhat revolutionary and not of general interest. They insisted that the ideas should only be published when there was definitive proof at a molecular level. Three years later, the concept of the metabolic basis of inherited disease became generally accepted when three monographs on the subject — those by Stanbury, Harris, and myself — all appeared within a few months of each other.

Today you are devoting a whole issue to the subject of "Inborn Errors of Metabolism". With this recognition, one can truly say that human biochemical genetics has become an integral part of clinical medicine.

David Yi-Yung Hsia, M.D.

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SUN LIFE OF CANADA

Hyperuricemia and Gout

A. Toguri, B.Sc. 6T9

INBORN ERRORS OF METABOLISM

The concept of inborn errors of metabolism as the etiologic basis of certain diseases originated from A. E. Garrod's work with phenylketonuria in which a distinctive clinical syndrome was discovered to follow a Mendelian autosomal recessive pattern of inheritance. Garrod, in the Croonian lectures in 1908, proposed that gout may be an inborn error of metabolism.[1] The rediscovery of Mendel's laws of inheritance by scientific circles enhanced this theory, but ironically, it too was forgotten and attention to it was focussed by G. W. Beadle forty years later when he articulated the one gene — one enzyme concept which was implicit in Garrod's writings.[2]

Tatum elaborated the relationship between metabolic abnormalities and genes in the following statements.[3]

(1) "All biochemical processes in all organisms are under genetic control.

(3) These biochemical processes are resolvable into a series of individual stepwise reactions.

(3) Each biochemical reaction is under the ultimate control of a different single gene."

The next statement was amended by Stanbury, Wyngarden, and Frederickson to include some new information.[4]

(4) "Mutation of a structural gene causes a change in structure of a specific protein and may affect the quantity of a limited number of other proteins. Mutation of a control gene alters the extent of function of one or more structural genes and therefore alters the amount of one or more proteins without changing their structure."

The investigators at the Pasteur Institute have done much in the field of molecular genetics of single cell systems; the concepts of regulator and operator genes hypothesized by Jacob and Monad to control or regulate biochemical pathways may be applicable to multicellular systems as well.[5]

It is therefore probable that particular biochemical abnormalities such as phenylketonuria, alpha or beta chain hemoglobinopathy, and hyperuricemia may be due to single or multiple defects. It will be in this vein that the etiology of primary gout and hyperuricemia will be discussed.

ETIOLOGY OF HYPERURICEMIA

Although it is difficult to evaluate hyperuricemia in a large population, the following are presented as evidence of the diverse genetic possibilities. C. J. Smith in 1948[6] showed that if prepuberal males were excluded from his population sample, the distribution curve of hyperuricemics corresponded to what one would expect if gout were an autosomal dominant type of inheritance. This data was recently reviewed by J. V. Neal[7] and found to be less convincing. Hauge and Harvald in 1955[8] have sufficient evidence to suggest that gout may be due to a polygenic inheritance. In the past year, Burch et al[9] studied the Blackfeet and Pima Indians and found that both geno-types (polygenic or autosomal dominant) may exist and that dietary factors were important.

The current thoughts of inheritance are probably best reflected by Stanbury, Wyngarden, and Frederickson[4]. They state that uric acid may be controlled by multiple genes and any one abnormality may exist and reveal itself as hyperuricemia in the population studied.

POSSIBLE MECHANISMS OF HYPERURICEMIA

The normal pathways of purine metabolism is shown in Fig. 1. The pathophysiology of hyperuricemia may be considered in terms of increased purine intake, decreased destruction, decreased excretion and increased production.

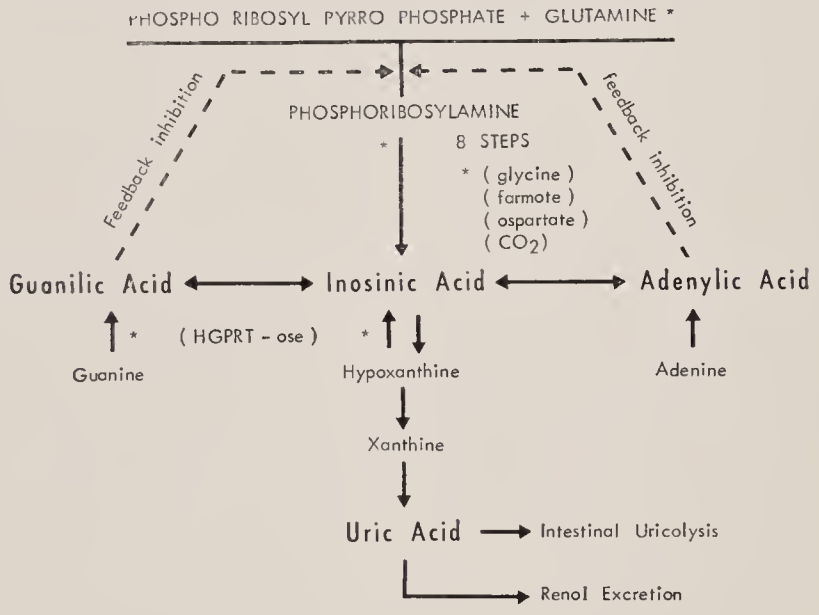


FIG. 1. Pathway of purine metabolism. Kindness of Dr. J. B. Houpt, New Mount Sinai Hospital, Toronto.

INCREASED INTAKE

It has been noted that serum uric acid will be lowered about 1 mg % by putting subjects on low purine diets; however, it may be raised by several mg % by putting subjects on a high intake of foods rich in nucleic acids.

Burch[9] has found diet to be important in the population he studied but others have shown diet to be an insignificant factor in gout.

DECREASED DESTRUCTION

Man, Dalmation dog and some anthropoid apes lack uricase, which in other species, degrades uric acid to

more soluble allantoin. Normally, about one third of the daily production of uric acid is excreted via the gut, where it is broken down by uricase produced by the intestinal flora. Decreased uricolysis via the gut is not a factor in the pathogenesis of primary gout. It may, however, be increased in primary gout or in the hyperuricema of renal disease.

DECREASED EXCRETION

The presently accepted view of renal handling of urate is that most of the filtered urate is absorbed and a fraction of this is secreted. It is possible that a genetic lesion expressed by a kidney abnormality may be a cause of gout. Proposed defects range from primary renal tubular pathology involving amino acid and urea metabolism to a generalized membrane transport disease.

To consider the former defect, it was noted by A. B. Gutman that some gouty patients with urinary pH less than 5.6 excreted less urinary ammonium than non-gouty subjects although the titratable acidity was the same.[18 19] He proposed that some gouty individuals had a deficiency of renal glutaminase which would result in shunting glutamine into the hepatic pathway for urate production. However, other workers have not found a decrease in renal glutaminase activity in their gouty patients.[21]

The latter hypothesis has not been investigated thoroughly because of the lack of techniques. Some workers have given urate loads to gouty and non-gouty individuals and have noted a failure in some individuals to respond adequately to the load.[29] Other workers have transplanted kidneys from a dog species having uricase to one lacking it. It was found that the changes thereafter in blood uric acid were unremarkable[11] More recent work on liver transplants between Mongrels and Dalmations show that the liver may play a significant role in urate handling.[12] This finding is interesting in view of the work done by Latham and Rodman which suggests that there may be a reduced affinity of a carrier substrate for urate or the presence of an inhibitor of uric acid excretion.[4]

The multiplicity of possibilities only stresses the diversity of mechanisms which may give rise to hyperuricemia by decreased excretion alone.

INCREASED URIC ACID PRODUCTION

Various mechanisms have been advanced to explain hyperuricemia. Some of these will be presented.

(a) *Increased Substrate levels*

The first site to be discussed occurs at what is thought to be the initial rate limiting step in normal purine synthesis (Figure 1). It has been shown that high levels of phosphoribosylpyrophosphate, glutamine, and glycine will increase uric acid. It is possible that in some hyperuricemics, there may be a genetic mutation increasing the production of these substrates.

(b) *Failure in Feedback Control*

A mechanism discovered to control the rate of production of adenylic acid (adenine-mono-phosphate) and guanilic acid (guanine-mono-phosphate) by feed back control of these purine-5- ribonucleotides can easily be fitted into the operon concept of Jacob and Monad, in terms of a cistron-polypeptide defect manifested by an enzyme giving rise to hyperuricemia. Glutamine phosphoribosylpyrophosphate amidotransferase is inhibited by adequate levels of purine -5- ribonucleotides as adenylic and guanylic acid and will turn off the production of inosinic acid (inosine monophosphate) or its immediate precursors. Failure to do this leads to increased de novo synthesis of inosinic acid and hence to uric acid (fig. 1). It has also been noted that low levels of these acids are more effective in inhibiting the enzyme than a comparable concentration of a single nucleotide. Hence, a deficiency of one of these nucleotides will likewise release inhibition of this pathway and so lead to hyperuricemia.[4]

Some clinical models manifesting specific abnormalities in purine synthesis are given.

(1) Lesch-Nyhan Syndrome — A distinctive clinical syndrome of self-destructive biting, hyperuricemia, mental retardation and choreoathetosis described by Lesch and Nyhan[13] has provided a model in which there is a total absence of hypoxanthine guanine phosphoribosyl transferase. This lack is thought to be genetic because of its familial incidence and compatibility with current concepts of intermediate metabolites.

(2) Another example of decreased enzyme activity is that reported by Kelley et al[14] who described two families in which hypoxanthine guanine phosphoribosyl transferase activity was decreased. The severity of this deficit was different between families but the same within families. It is important to note that there are other hyperuricemic families with normal levels of this enzyme.

In concluding, the seemingly confusing and conflicting reports are interpreted by many investigators as proof of the wide spectrum of genetically controlled mechanisms giving rise to the phenotypic expression of hyperuricemia and clinical syndrome of gout.

CLINICAL ASPECT OF GOUT

Hyperuricemia or an elevated blood uric acid is not pathognomic of gout although those who manifest an acute gouty attack or chronic gout usually have hyperuricemia. This means that the individual's serum uric acid is two or more standard deviations above the mean of the population studied by a particular method. The average in most laboratories is 6 mg % in postpuberal males and postmenopausal females and about 5 mg % in

postpuberal but premenopausal females. There appears to be a wide range of serum concentrations at which point people will manifest an acute episode of gout. The 24-hour urinary excretion of uric acid in the adult male varies from 300 to 500 mg and in gouty persons it varies from 200 to 1500 mg per day.

The differential diagnosis of hyperuricemia includes primary hyperuricemia, secondary hyperuricemia due to polycythemia, leukemia, lymphomas, chronic renal insufficiency, Benzothiadiazine drugs, ASA, obesity, starvation, sarcoid, juvenile gout, extensive psoriasis and glycogen shortage disease type 1.[4]

The incidence of hyperuricemia seems to vary widely and appears to depend on geographical and racial factors. The surveys done in North America manifest the difficulty in determining the incidence of primary gout in such a heterogenous population. This is a disease of adults, although it may occasionally occur in children, being more prevalent in men than women and being more common in postmenopausal females than premenopausal ones. It is most frequent during the third to fifth decades.

ACUTE GOUTY ARTHRITIS

The precipitating factors, nature of onset, pain, and clinical course were well described by Sydenham in 1683[15], "The victim goes to bed and sleeps in good health. About two o'clock in the morning, he is awakened by a severe pain in the great toe, more rarely in the heel, ankle or instep. The pain is like that of a dislocation, and yet it feels as if cold water were being poured over them. Then follow chills and shivers, and a little fever. The pain, which at first is moderate, becomes more intense. With its intensity, the chills and shivers increase. After a time, this comes to a height, accommodating itself to the bones and ligaments of the tarsus and metatarsus. Now it is a violent stretching and tearing of the ligaments — now it is a gnawing pain and now a pressure and tightening. So exquisite and lively, meanwhile is the feeling of the part affected, that it cannot bear the weight of the bedclothes nor the jar of a person walking in the room. The night is passed in torture, sleeplessness, turning of the part affected, and perpetual change of posture; the tossing about of the body being as incessant as the pain of the tortured joint, and being worse as the fit comes on. Hence, the vain effort, by changing of posture, both in the body and the limbs affected, to obtain abatement of the pain."

Although Sydenham's description is vivid, one must rule out pyogenic arthritis, acute episodes of rheumatoid arthritis, adult rheumatic fever, acute Heberden's nodes, and chondrocalcinosis (pseudogout). Some aids to diagnosis are a therapeutic trial of colchicine and a joint aspiration to recover mono-sodium urate crystals.

The natural course of this disease after a single acute attack is usually spontaneous recovery in a few days to weeks with recurrence in a few months to years. The frequency and duration of the following attacks increase.

MECHANISM OF AN ACUTE ATTACK

Acute gouty arthritis is thought to arise from the crystallization of monosodium urate in the articular cartilage or synovium due to the hyperuricemia of body fluids and local factors. The crystals incite local inflammation which activates Hageman factor and kinin forming enzymes in the joint fluid as well as inducing a leukocytosis. The inflammatory cells phagocytose the crystals and the metabolic activity is manifested by a drop in pH due to an increase in lactic acid. The decrease in pH reduces the solubility of sodium urate and hence aids in crystallization. The cells degenerate releasing their lysosomes and kinins which tend to destroy the mucoproteins leading to further inflammation, decrease in pH and further crystallization.. Hence, the cycle repeats itself.[16]

Colchicine is thought to stop this cycle of events and so decrease inflammation. The concepts presented to explain its mode of action are thought to be one or more of the following: (1) It interferes with the production of lactic acid by phagocytes by decreasing their metabolism. (2) It inhibits chemotaxis of normal leukocytes and/or (3) it inhibits amoeboid mobility of leukocytes. The effects of all these will block the cycling mentioned above.

CHRONIC GOUTY ARTHRITIS

The onset of this may be insidious or may be heralded by many attacks of acute gout. Chronic gouty arthritis is manifested by tophaceous deposits in one or more sites as in the great toe, instep, ankle, heel, knees, wrists and ears. Its natural course causes destructive and deforming lesions. The tophi may painlessly ulcerate and drain a chalky exudate. This form of primary gout should be differentiated from rheumatoid arthritis.

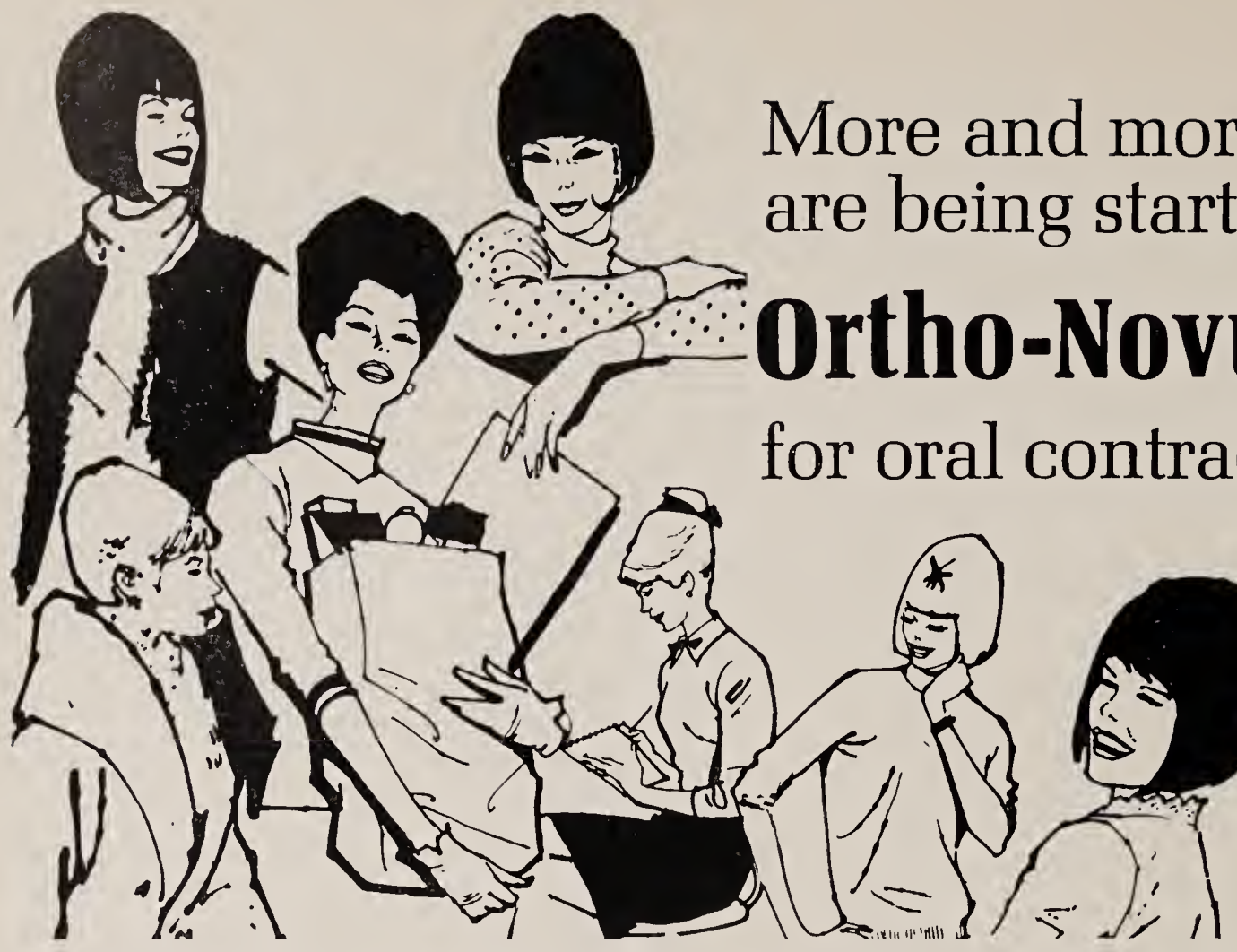
PATHOLOGY

The pathognomic lesion is the multicentric needle-like crystals of sodium urate surrounded by inflammatory tissues and a foreign body reaction. The urates are deposited in the cartilage, epiphyseal bones, and peri-articular structures. Here, in addition to the general response noted above, there may be degeneration of cartilage and subchondral tissue, synovial and marginal bone proliferation, development of fibrosis and bony ankylosis.

Radiographically, these deposits may appear as punched out lesions or seldomly, as calcified deposits peripherally.

ASSOCIATED PATHOLOGY

Cortical and renal pelvic pathology are seen in hyperuricemia. Early changes are glomerulosclerosis with uniform fibrillar thickening of the glomerular capillary base-



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ment membrane which is regarded as distinctive. Later changes are: glomerular hyalinization, vascular hypertrophy, interstitial fibrosis and inflammation. Hypertension associated with this is usually benign. Uric acid stones tend to form in approximately 20% of gouty people and 40% of overproducers of uric acid.

IN SUMMARY

Information was presented showing the multitude of mechanisms which may explain the genetic basis of primary gout.

In addition, the clinical features of gout were briefly reviewed:

(1) family history of gout;

(2) clinical course and features — acute monarticular or polyarticular arthritis with or without tophi;

(3) laboratory finding of persistent hyperuricemia;

(4) presence of monosodium urate crystals in tophi.

ACKNOWLEDGEMENT

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Phenylketonuria

M. Rynne 7T1

A small but significant number of infants are born each year with phenylketonuria, a common form of "inborn errors of metabolism". Phenylketonuria (PKU) is an inherited disease in which mental retardation of the individual is associated with increased amounts of phenylalanine and abnormal phenylalanine metabolites as described by Folling in Germany in 1934. The disorder results from a decrease in the activity of phenylalanine hydroxylase, an enzyme that converts phenylalanine to tyrosine (fig. 1).

The disorder accounts for approximately 5% of institutionalized mental retardates. Improved mass screening procedures and dietary therapy should drastically reduce this figure in the next generation.

A. Genetics

The lesion resulting in PKU is transmitted as an autosomal recessive gene with a frequency of about 1/20,000. Within the family where each parent is a heterozygote a one in four chance of bearing a phenylketonuric child exists. Two out of four children would be carriers. Carriers are present in the population with a frequency of one in fifty.

The gene causing PKU has been related to the third allele of the enzyme phenyalanine hydroxylase. Biochemical kinetics have demonstrated altered affinity for the substrate, phenylalanine, in the heterozygous and homozygous recessive individual.[1]

Families with known histories of inborn errors, especially PKU must be counselled about the possible consequences on their yet unborn offspring.

B. Pathophysiology

The inability to metabolize phenylalanine to tyrosine is due to a defect in the activity of the enzyme, phenylalanine hydroxylase, in the liver. Abnormal amounts of phenylalanine metabolites appear in the blood and are sometimes excreted in the urine. These metabolites are phenylpyruvic acid, phenyllactic acid, phenylacetic acid, and o-hydroxyphenylpyruvic acid. The last metabolite is present in the PKU child but not the normal. Note that small amounts of phenylpyruvate and phenyllactate are probably present in normal tissue.

The excessive accumulation of these metabolites apparently prevents normal development of the nervous system, particularly the brain. Neuropathological reports describe the presence of multiple areas of impaired myelination in the adult phenylketonuric.

C. Detection

Because of the genetics of phenylketonuria it is advisable that each neonate be screened at certain points in his development for abnormal phenylalanine metabolites. This is especially true if the family presents a history of phenylketonuria *or* undiagnosed mental disease.

Laboratory methods for screening and diagnosis include urinalysis and serum tests. However, for continuous monitoring, the serum analysis is the *only* satisfactory method.

Abnormally high plasma levels of phenylalanine occur 2 to 4 days after milk feedings begin i.e. at about 4 - 6 days after birth. Phenylketone compounds are excreted into the urine only after their concentration reaches a level of 10 - 15 mg/100 ml blood. This occurs between 10th and 14th day.

It has been recommended that tests should be started no sooner than 24 - 36 hours after the initial milk feeding. If the family history indicates previous problems, daily analysis should be carried out until the child is discharged. If results were negative, tests at the fourth and sixth week are indicated. This would be done to diagnose late developing phenylketonuria. Extreme care must be exercised in differentiating PKU from other causes of hyperphenylalanemia.

CRITERIA FOR DIAGNOSIS OF PKU

I Abnormal metabolites of phenylalanine present in serum and urine; (normal phenylalanine 1 - 3 mg/100 ml in serum).

II Normal tyrosine levels 1 - 3 mg/100 ml.

III Significantly elevated fasting phenylalanine levels 10 mg/100 ml or above. Phenylalanine assays of 4 mg/100 ml or more should be repeated promptly as suggestive but not conclusive.

IV Presence of o-hydroxyphenyl pyruvic acid.

Quick, inexpensive and easily performed, urine tests such as the *Ferric Chloride Test* are good for the screening of large populations. Since the transport mechanism in excretion is only operative at levels between 10 to 15 mg/100 ml and above, higher than normal levels may exist in the plasma without detection in the urine. Also the inherent variability of excretion of phenylketone metabolites will result in only eighty per cent positive urine tests, i.e. 20% false negative test results.

METHOD

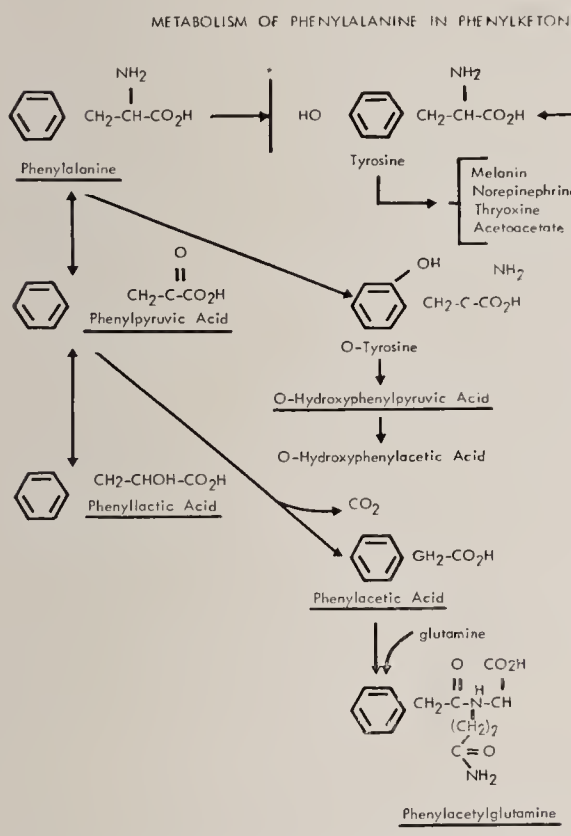
Add three drops of 10% ferric chloride solution to one ml of urine.

Interpretation: Immediate appearance of medium dark blue-green to gray-green color indicates the presence of phenylpyruvic acid and/or other phenylketone com-

DIFFERENTIAL DIAGNOSIS OF DISORDERS OF PHENYLALANINE AND TYROSINE METABOLISM

Clinical State	*Serum Amino Acids*		*Urinary Amino Acids*			*Urinary Phenols*	
	*Phe**	*Tyr**	*Phe*	*Tyr*	*Others*	*Orthohydroxy-phenylacetic Acid*	*Parahydroxy-phenylacetic Acid*
PKU (untreated)	++++	+	++++	+	+	++++	+
New born of untreated PKU mother: 1st day (infant not PKU)	+++	+	+	+	+	+	+
Transient tyrosinemia (full-term premature infants)	++	++++	+	++++	+	0	+++
Permanent tyrosinosis (enzymopathy)	+	++	++	++++	++++	0	+++
Normal	+	+	+	+	+	0	0
"Hyperphenylalaninemia"	++	+	+	+	+	±	0

*Phe is phenylalanine and tyr is tyrosine.



*Phenylketonuria is characterized by absence of a liver enzyme, phenylalanine hydroxylase, which results in a metabolic block in the conversion of phenylalanine to tyrosine.
NOTE: The underlined metabolites appear in the urine.

FIG. 1. Metabolism of phenylalanine in phenylketonuria.

pounds. Positive tests may also indicate the presence of homogentisic acid of alcaptonuria, catecholamines of pheochromocytoma, or maple syrup urine disease. Salicylates will give a red-burgundy color in ferric chloride and have been known to cause false positives. A variety of methods carried out on diapers and filter paper wetted with urine are available.[6] Phenistix (Ames Co.) are commonly used to test for presence of phenylketones in urine. They are simple and probably more accurate than ferric chloride.

Another more analytical procedure is the *Guthrie Inhibition Assay technique.*[7] It is used on serum *or* urine. The mechanism depends on disinhibition of growth of B. Subtilis seeded with B-Thienylalanine. It is more sensitive than the ferric chloride test — with a level of detection at 1 mg/100 ml. A positive test is considered to be any level above 6 mg/100 ml.

Fluorometric analysis and paper chromatographic techniques have both been developed. However, the ferric chloride and Guthrie are two of the more common tests in use.

Positive tests should be subjected to more quantitative evaluation. This will facilitate the planning of the diet.

Differential Diagnosis

With larger populations being screened and with more refined tests being performed, a number of closely related diseases have been described. It is assumed that high phenylalanine levels would remain so, but Hsia reports a number of variants on this problem which question and in part refute this.[9] Consequently, the benefits and hazards of early phenylalanine restriction must be cautiously weighed against the possibility that such a patient may develop normally without a low phenylalanine diet. Some examples are:

(1) Hyperphenylalanemia with tyrosinemia: This is due to decrease tyrosine transaminase and parahydroxyphenylpyruvic acid oxidase. There is a moderate increase of phenylalanine with a marked increase of tyrosine. Seldom does the level exceed 10 mg/100 ml. Demonstrations have shown that if there is an increase of phenylalanine there is an increase of tyrosine.

(2) Transient Hyperphenylalanemia: Similar to findings in PKU, there is a delay in the maturation of the phenylalanine oxidase system. This occurs in about

twenty-five per cent of premature infants and a small percentage of full term newborns. If diet were to be applied in such a case, the development of the child might be handicapped considerably.

(3) Persistent Hyperphenylalanine: High levels without the phenylketonuric retardation have been reported. This is due to a decrease but not absence of phenylalanine hydroxylase found by liver biopsy.

Thus variations in reports of the frequency of PKU depend on the discrimination of those various types of hyperphenylalanemia. Consequently, Hsia has recommended that a more rigid set of criteria be applied before diet is initiated. These include Phenylalanine Tolerance Tests for the immediate family, and if indicated, a liver biopsy.

Further institution of diet would be dependent on levels of phenylalanine rising above 20 mg% with normal tyrosine levels. At intervals of three, six and twelve months, the child is removed from diet until serum phenylalanine rises above 20 mg%. Such precautions are necessary in light of the reanalysis of populations of phenylketonurics who were not of the classical type, but were treated as such.

E. PKU Management

Management requires the close co-operation of the whole health team esyecially the biochemist and dietitian. These disciplines work on the process of titrating the balance between phenylalanine levels and developmental requirements. A safe range was considered to be between 2 and 7 mg/100 ml of phenylalanine. Problems arise from the excess of these metabolites. Dietary control must be established early and monitored closely if it is to be successful. The critical time period is within the first two months of life. Most successful treatment, therapy to minimize retardation, has been initiated during this interval.

All natural food proteins contain about five per cent phenylalanine. The rationale for the diet is to reduce this intake. It can be accomplished by the consumption of a specially prepared food from which a significant amount of phenylalanine has been removed. Mead-Johnson currently provides Lofenalac, a casein hydrolysate preparation to supply dietary nitrogen in the form of amino acids and small peptides. Carbohydrates and fats are added to supply calories.

It must be remembered that phenylalanine is an essential amino acid in human nutrition. Adequate but limited amounts of the acid must be supplied for growth and development in infants and children affected by PKU.

Plasma levels of phenylalanine should be monitored weekly in the early stages with the interval increasing to monthly when the child is well controlled. Other parameters of growth such as bone growth and elongation, weight gain, must also be considered. The balance of these physical conditions and the mental development of the child is the essence of treatment.

Foods such as fruit, cereal and vegetables, which contain little protein are usually added to supplement the dietary phenylalanine and to provide variety.

Plasma phenylalanine levels between 3 - 7 mg/100 ml are recommended for two reasons. It is lower than the lowest level of reported retardation due to phenylketonuria and it is above the catabolic threshold mechanism for protein. Levels of 8 - 15 mg% have been associated with permanent and irreversible damage. The usual range for the untreated phenylketonuric is 20 - 60 mg/100 ml of serum.

F. Clinical Aspects

Early symptoms of phenylalanine deficiency include anorexia, fever and lethargy. Unless treatment is initiated, epileptic seizures may result. Reversal may be accomplished by the addition of small amounts of milk *or* protein to the diet.

Febrile illnesses are often characterized by elevations in plasma phenylalanine with increasing urinary phenylalanine and appearance of ortho-hydroxy-phenylacetic acid in the urine. Changes are thought to be due to protein catabolism. Good control can be achieved by liberalizing protein intake with jello, soups, broths or small quantities of milk. Monitoring of plasma and urine levels is essential.

The phenylketonuric child and adult usually have blond hair, blue eyes, a light complexion and a peculiar musty odour. Eczema is observed in about 20% of cases. Seizures occur in twenty-five per cent while seventy to eighty per cent have abnormal EEG patterns, with spike and wave complexes in the more extreme cases.

At birth the child appears normal with normal progress within the first three months. However early signs displayed are:

(1) intolerance to milk feeding
(2) regurgitation
(3) failure to gain weight

Delayed ability to sit may be the first of conspicuous signs. In the extreme case the affected child may not walk until early childhood, or not at all.

In the second three months, the child may appear listless, apathetic, and fail to respond to parents. In summary, one may see a general slowdown in the developmental sequence. In the older retarded child one would observe: extreme hyperactivity, irritability and destructive tendencies, a lurching and high stepping gait, unusual posturing of hands and fingers, autistic behavior, resulting in an expressionless preoccupation with space. Speech may be underdeveloped; however, normal intellectual function may be reached. Negativism, apprehension and emotional outburst may occur.

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In some cases the diet relieves the irritability and tension. However, the application of the diet to adults in order to improve eczema and irritability does not seem advisable. Diets in the home seem more successful than those in institutions.

In summary, PKU was presented, giving its aetiology as an inborn error of metabolism. Stress was placed on the continuous monitoring of a phenylketonuric patient using laboratory techniques and multidisciplined personnel. It was also noted that all hyperphenylalanemics should *not* be treated with diet.

ADDENDUM

Referral of Patients: The Province of Ontario PKU screening system provides three centers for the treatment of the positive test producing child.

If a positive test with the Guthrie Inhibition Assay is recorded, referral to one of the three centers for further investigation is recommended. Also recommended is a ferric chloride test *or* Phenistix in the fourth and eight weeks.

If the child is diagnosed as a PKU and is registered with the Ontario Department of Health, dietary requirements are available, free of charge from one of the three PKU centers. Diets should be supervised from one of those centers.

Locations of the centers:

London War Memorial Children's Hospital
Kingston General Hospital
Toronto Hospital for Sick Children

The child may also be referred through the Maternal and Child Branch of the Dept. of Health.

ACKNOWLEDGEMENT

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The Thalassemic Syndromes

B B. Henry 6T9

In 1925 Cooley and Lee in Detroit described a chronic progressive anemia occurring in Italians and other peoples from the Mediterranean Basin. It is characterized by onset in the first year of life, by splenomegaly, and by microcytic hypochromic erythrocytes, poikilocytes, erythroblasts and target cells in the peripheral smear.[1] This anemia is known as Mediterranean Disease, Cooley's Anemia, Target Cell Disease, and Thalassemia. Genetic studies demonstrated that the parents of children with Cooley's Anemia frequently had a less severe but qualitatively similar anemia.[2] Consequently, it was proposed that Cooley's Anemia was the homozygous phenotype of an inherited anomaly of erythrocytes. The anomaly is manifested in the heterozygote by a longer life, milder symptoms and a less abnormal blood picture. But the hemoglobin electrophoresis has shown that Thalassemia is not a single disease but a group of diseases characterized by quantitative abnormalities of hemoglobin.

In 1957 it was proposed that quantitative deficits of specific globins cause the Thalassemic Syndromes and that these deficits are due to inborn errors of globin synthesis.[3]

Because of the high incidence of the Thalassemias among immigrants from the Mediterranean Basin, and their descendants, these quantitative hemoglobinopathies are the commonest cause, after iron deficiency, of hypochromic microcytic anemia in North America.[2]

On the basis of different globin content, normal hemoglobin is composed basically of three hemoglobins: A, A_2, and F. Hb A contains alpha and beta globins, Hb A_2 contains alpha and delta globins, Hb F contains alpha and gamma globins (two chains of each type [4 in all] combined to heme). These globins are structurally distinct polypeptides and the synthesis of each type is mediated by separate autosomal alleles.

Table 1. Percent of Hb Fractions in the Newborn and Adult.

	Newborn	*Adult*
Hb A (alpha 2, beta 2)	10 - 40	96 - 98
Hb A_2 (alpha 2, delta 2)	0.5 - 1.5	1.5 - 4.0
Hb F (alpha 2, gamma 2)	60 - 90	0 - 3.0

The rate of synthesis of each globin chain can be estimated by labelling hemoglobin in reticulocyte incubations in vitro with radioactive amino acids, by separation of alpha and beta chains of human globin from hemoglobin, and finally, by measuring the radioactivity in alpha chains and beta chains.[4] Whereas alpha and beta globins are synthesized at the same rate in normal reticulocytes, one of these globins is synthesized relatively slower in thalassemic reticulocytes.[5] The Thalassemias are classified according to which globin is synthesized in inadequate amounts: alpha, beta, delta and gamma Thalassemias.

In beta Thalassemia the rate of beta globin synthesis is decreased. Because beta globins are essential constituents of Hb A, Hb A synthesis and the Hb A fraction of blood Hb are decreased.

Since Hb F and Hb A_2 do not contain beta globins, their formation is unimpaired and the Hb F and Hb A_2 fractions of blood hemoglobin are relatively increased.

Although gamma chain synthesis usually decreases after three months of age, it is sustained in beta Thalassemia. The total number of beta and gamma chains synthesized is normally equal to the number of alpha chains produced if the number of delta chains produced is assumed to be negligible. In beta Thalassemia the total number of beta and gamma chains formed is usually less than one quarter of the number of alpha chains synthesized. Because alpha and beta chains are utilized in equal numbers in hemoglobin A formation, the decreased number of beta chains is manifested as a decrease in the amount of Hb A synthesized. Since gamma chain production does not compensate for the decreased beta chain production, the total hemoglobin is decreased although the Hb F (alpha 2, gamma 2) fraction is increased and the uncombined alpha chain pool is increased.[6] Free alpha chains are chemically unstable and precipitate in the cell stroma forming inclusion bodies which seem to predispose the beta Thalassemic erythrocyte to premature destruction.[6 7]. Beta Thalassemias are most commonly associated with the clinical picture of Cooley's Anemia. Studies have shown that different amounts of beta globin are synthesized in different beta thalassemic populations. This suggests that the cause of beta Thalassemia is not one, but a heterogenous group of mutations of the operator genes controlling beta globin synthesis. The clinical picture is severe if the mutation is homozygous for an operator allele.

In alpha Thalassemia, alpha globin synthesis is defective so that Hb A, A_2, and F are all affected. If the fetus is homozygous for two alpha thalassemic genes, hydrops fetalis and in utero death result; without alpha globins, the gamma globins form stable tetramers (hemoglobin H) which can be detected in cord blood. Because these tetramers bind oxygen irreversibly, the fetus becomes anoxic and dies.[8] In the heterozygous type a milder deficit of alpha globin occurs and because 5 - 30% of the hemoglobin formed is Hb H, it is called Hb H disease. Hemoglobin H precipitates in older erythrocytes and inclusion bodies. The fact that inclusion body containing cells are only found in peripheral blood after splenectomy in this disease, infers splenic removal of such cells from circulating blood.[7] Neither defective synthesis of delta or gamma

globins are clinically important, the former because it affects only the minor hemoglobin component A_2, and the latter because it is normally produced only in small amounts after the first three months of life.

PATHOGENESIS

The anemia is due to 1) defective hemoglobin production and 2) hemolysis. Decreased globin synthesis causes a decrease in globin chains available for incorporation into hemoglobin. The other subunits of hemoglobin (Fe, protoporphyrin, heme and unaffected globin chains) accumulate. Fe 59, C14 — glycine and C 14 — protoporphyrin incorporation into thalassemic reticulocytes in vitro are consistently lower than in normal control reticulocytes. This is interpreted to represent two possible blocks in heme production: i.e., between succinyl-Co-A and glycine conversion to delta-aminolevulinic acid, and between protoporphyrin and heme. The accumulation of heme might cause feedback repression of heme-synthesizing enzymes.[9] Serum iron is elevated, iron binding capacity is negligible and hemosiderosis occurs. Protoporphyrin breakdown elevates serum bilirubin.

Hemoglobin-poor erythrocytes and inclusion body containing cells are lysed prematurely. In beta Thalassemia, intramedullary hemolysis occurs, because unstable alpha globins precipitate in the stroma before the erythrocytes enter the circulation. In alpha Thalassemia more stable beta globins form tetramers and inclusion bodies only in older erythrocytes so that the erythrocyte half life is longer than in beta Thalassemia. Inclusion bodies probably predispose to erythrocyte destruction and the following mechanisms may apply: by causing membrane damage, ion leakage occurs and subsequently overworks the sodium pump. Membrane integrity may be prejudiced by depletion of available SH groups either by direct attachment of precipitating globins composing the inclusion bodies, or by their binding glutathione during precipitation. Thalassemic cells have a high rate of glycolysis with a low and unstable ATP content; possible mechanisms are mitochondrial dysfunction due to excessive iron deposition or increased ATP demand by the accelerated sodium pump.[6]

Thus shortened erythrocyte survival and anemia are primarily due to an imbalance in globin synthesis and secondarily to hemolysis. The fact that the magnitude of the excess alpha chains compared to beta chains in beta Thalassemia correlates well with Cr^{51} labelled erythrocyte survival, provides more evidence of the importance of the imbalance of chain production as the basic cause of hemolysis.[10]

THE NATURE OF THE DEFECT

An early hypothesis stated that Thalassemia resulted from an inherited amino acid substitution on the globin chain which did not change the electrophoretic mobility but decreased its rate of synthesis. That thalassemic globins are known to be qualitatively normal has discounted this theory.

There may be absence of part of the chromosome bearing all or part of the locus responsible for the encodement of RNA for a subunit polypeptide. This could be due to deletion during cross-overs between non-homologous chromosomes.

Radioactive uptake studies on beta thalassemic reticulocytes, estimating the distribution of radioactivity in pure hemoglobin and in growing chains on ribosomal fractions, suggest that the delay in beta globin assembly is at the ribosomal level.[4] Because other globins are produced normally, the deficit in beta Thalassemia must involve messenger RNA for beta globin. This hypothesis is supported by the observation that thalassemic reticulocytes respond normally to artificial RNA (poly u).[11] The messenger RNA is quantitatively and/or qualitatively abnormal. The latter might be due to an alteration of a base such that a transfer RNA which is required to insert the appropriate amino acid is in short supply. Alternatively, the miscoding due to substitution of a base on DNA might result in defective chain initiation or termination so that globin synthesis is slowed. The attraction of this theory is that it permits many different sites in the delay of beta globin assembly and would partially account for the variable severity observed clinically.[12]

CLINICAL FINDINGS

The spectrum of severity is broad so that the terms Thalassemia major, intermedia, minor and minima have been utilized. Severity is generally inversely proportional to age of onset and directly proportional to the number of thalassemic allele mutations present. The incidence in persons of Italian descent is 1/2368 births in the severe form and 1/25 births in the mild form.

Although Thalassemia occurs mainly in peoples of the Mediterranean countries it is also found in India, China, Malaysia, Africa and the West Indies.[8]

Classical Cooley's Anemia is progressive and severe, commencing between the third and sixth month of life. Splenomegaly and hepatomegaly, cardiac dilatation and failure are common. X-rays of skull show thickening of the diploe and "hair on end" appearance of striations between the tables. The long bones show a thin cortex due to marrow hyperplasia. The erythrocyte count is usually decreased. The smear shows normal platelets, mild leukocytosis with occasional immature myeloid forms, microcytic hypochromic erythrocytes, poikilocytes, erythroblasts and occasional nucleated erythrocyte and target cells.

Homozygous beta Thalassemia is fatal in the first year in untreated cases, death being due to intercurrent infection or cardiac failure secondary to iron deposits in the myocardium.

As noted previously the milder forms are more common in adolescents and adults and are asymptomatic or demonstrate only increased fatigue. Splenomegaly and icterus are moderate and the morphologic changes in the erythrocytes are out of proportion to the degree of anemia. Prognosis for a long life is excellent although intercurrent infection may be associated with aplastic crises, and pregnancy can aggravate the anemia probably by increasing the requirements for folic acid and vitamin B_{12}.

Routine hematologic studies are augmented by hemaglobin electrophoresis on paper or starch gel, alkaline denaturation of erythrocytes to demonstrate elevated Hb F fraction, osmotic fragility determinations, and cresyl blue incubations and examination for inclusion bodies. By combining these tests with the family and racial history, the diagnosis is reached.

The Thalassemias are differentiated from iron deficiency by the high serum iron, normal iron stores in the marrow, and the resistance of the anemia to therapeutic iron; target cells and cells showing punctate basophilia are more common in the former than in the latter.

MANAGEMENT OF THALASSEMIA

Therapeutic tools presently available are:

1) Whole blood transfusions
2) Antibiotics
3) Folic acid supplements
4) Chelating agents

It is not clear whether children with Thalassemia major have a better prognosis if maintained with hemoglobin levels of 8 - 9 gm% or at completely normal levels by frequent blood transfusions; the latter situation is associated with better growth and all round development but leads to generalized siderosis. The most serious problem in these children is cardiac failure secondary to massive myocardial iron deposition.

Hemoglobin levels over 10 gm % have been maintained by transfusion, using chelating agents concomitantly to minimize the increase in iron loading (DFOX or desferrioxamine methane sulfonate; DTPA or trisodium diethylene triamine pentacetate). This regimen caused marked improvement in physical activity, reduced heart size, and eliminated all cardiac symptoms, eg. exertional dyspnea and angina, and provided at least short term clinical benefits in severely affected children.[13]

Splenectomy is reserved for the following instances:

1) where splenic compression of other structures causes distress;

2) hypersplenism as judged by leukopenia and persistant thrombocytopenia;

3) excessive hemolysis or splenic pooling as judged by Cr^{51} studies associated with splenic scan.

Only the "pure" Thalassemias have been described and this has been done in simple terms. The Thalassemic variants, eg. Hb Lepore, and the mixtures combining Thalassemic defects with a qualitative hemoglobinopathy, eg. Hb S, are noted only in passing. For classification by detailed electrophoresis and discussion of the variant and mixed Thalassemic Syndromes, the references may be consulted.

SUMMARY

The Thalassemic Syndromes are genetically determined anemias. Decreased rate of globin synthesis causes decreased formation of specific hemoglobin fractions and premature erythrocyte destruction. Presently the wide range of clinical pictures is being correlated with the results of hemoglobin electrophoretic and biochemical studies. Defective globin synthesis seems to involve messenger RNA at a ribosomal level. It is likely that either there is a total or partial reduction in the rate of production of messenger RNA for the affected globin, or the messenger RNA is quantitatively normal but is qualitatively abnormal so that globin chain assembly on the mRNA-ribosomal template is retarded.

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Detection Of Inborn Errors Of Metabolism In Newborns

M. Waxman 6T9

In recent years wide-scale screening programs of patients with mental retardation or diseases of unknown origin have demonstrated many new "inborn errors of metabolism". These diseases are caused by the inactivity or absence of a specific enzyme required for normal metabolic activity. The correct diagnosis can be established by appropriate biochemical tests even though no clinical evidence of the underlying metabolic disorder is present.

The main difficulty in diagnosing these diseases lies in deciding which patients merit the detailed and often expensive laboratory investigations necessary to identify the exact biochemical defect. Therefore, it is necessary to have a program of screening tests which ideally should be simple, efficient, reliable, sensitive and economical. In this way, abnormal metabolites may be detected and patients selected who should be investigated further.

Very recent evidence has revealed that biochemical abnormalities of some of these diseases, for example, phenylketonuria and galactosemia, can be detected by examination of a sample of amnionic fluid surrounding the fetus in utero. In this procedure enzymatic defects are found in the amnionic cells. However, since amniocentesis is not a routine procedure at present, biochemical abnormalities are not usually detected until after birth.

It is of utmost importance to detect affected patients in early life since many of these disorders cause severe damage. With early detection and appropriate therapy such dire consequences as mental retardation may be prevented. Even though no treatment has been proposed for many of these metabolic disorders, recognition of their etiology can be of great importance in prognosis, management and genetic counselling.

Hsia[1] has presented four principal approaches to screening for hereditary metabolic disorders: 1) use of bacterial "inhibition assay" in several specific conditions; 2) reduction of nicotinamide adenine dinucleotide phosphate (NADP) in disturbances of erythrocyte metabolism; 3) detection of amino acids in the blood and urine by chromatographic methods; 4) other specific methods. I will employ these four principal approaches in the following discussion of detection of inborn errors of metabolism in newborns.

BACTERIAL "INHIBITION ASSAY"

In 1963, Guthrie and Susi[2] described a semiquantitative method for detection of elevated phenylalanine levels among newborn infants before they were discharged from hospital. The test is based on the principle that the inhibition of growth of bacillus subtilis by beta-2-thienylalanine in a minimal culture is specifically prevented by proline, phenylalanine, phenylpyruvic acid, or phenylacetic acid. A small amount of fresh blood obtained by skin puncture is transferred immediately to a piece of filter paper. The blood spot is dried, autoclaved, punched out and placed over the agar surface containing inoculum and the inhibitor and the results are interpreted sixteen hours later. A halo surrounding the disc represents growth of the organism and indicates an increase in phenylalanine content which is relative to the size of the halo. This principle has been extended to detect other diseases of amino acid metabolism such as Maple Syrup Urine Disease and Histidinemia.

For galactosemia, the test is based upon a different principle. Here the test is directly carried out with Escherischia coli, strain W3101, a mutant which is galactose sensitive and produces no uridyl transferase. When blood containing an excess of galactose is used the growth of bacteria is inhibited because the galactose which enters the bacterial cell is converted by galactokinase to galactose phosphate which then accumulates. Thus a diagnosis of galactosemia is suggested when the bacteria do not multiply.

TABLE 1[1] Bacterial "Inhibition Assay"

Condition	*Principle*
1. Phenylketonuria	phenylalanine prevents inhibition of B. subtilis by beta-2-thienylalanine
2. Maple Syrup Disease	leucine prevents inhibition of B. subtilis by 2-methyl leucine
3. Histidinemia	histidine prevents inhibition of B. subtilis by azaseline
4. Galactosemia	galactose inhibits growth of E. Coli strain W3101

REDUCTION OF NADP

A failure to reduce NADP has been used to detect three enzyme defects in erythrocytes: glucose-6-phosphate dehydrogenase deficiency, pyruvate kinase deficiency, and glutathione reductase deficiency.

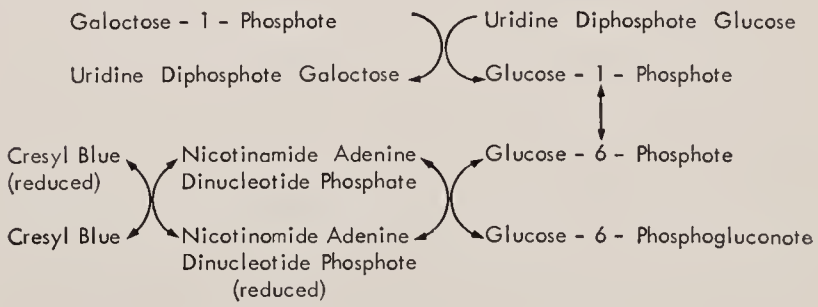
Galoctose - 1 - Phosphote
Uridine Diphosphote Glucose
Uridine Diphosphote Galoctose
Glucose - 1 - Phosphote
Glucose - 6 - Phosphote
Cresyl Blue (reduced)
Nicotinamide Adenine Dinucleotide Phosphate
Cresyl Blue
Nicotinomide Adenine Dinucleotide Phosphote (reduced)
Glucose - 6 - Phosphogluconote

FIG. 1. Decolourization test for detecting deficiency of glucose-6-phosphate dehydrogenase.

TABLE 2 *Some Inborn Errors of Metabolism Characterized by Elevated Blood Amino Acids:*[5]

Disease	*Amino Acid Accumulated in Blood*	*Chief Clinical Features in Patients with Biochemical Defect*
Phenylketonuria	Phenylalanine	Mental retardation Convulsions Eczema Fair hair & skin
Maple-syrup urine disease	Valine Leucine Isoleucine Methionine	Mental retardation Spasticity Myoclonic seizures
Homocystinuria	Methionine Homocystine Homocysteine-cystiene disulfide	Mental retardation Seizures Dislocated lenses Thromboembolic
Tyrosinosis	Tyrosine	Myasthenia gravis
Histidemia	Histidine	Delayed speech development Mental retardation

In 1961, Motulsky and Campbell-Kraut described a simple decolourization test for detecting a deficiency of glucose-6-phosphate dehydrogenase (G-6-PD). As shown in *figure 1*, when glucose-6-phosphate is converted to 6-phosphogluconate, NADP is reduced to NADPH. When NADPH is oxidized, the dye brilliant cresyl blue is reduced and decolourization occurs. In the normal subject with adequate G-6-P D, decolourization occurs in forty to sixty minutes, while in the individual with a deficiency of G-6-P D decolourization may not take place for ninety minutes to twenty-four hours.

In 1964, Beutler et al[3] extended this test to detect deficiency of galactose-1-phosphate uridyl transferase in galactosemia.

In 1965, Beutler[4] took advantage of the fact that even small quantities of reduced pyridine nucleotides fluoresce intensely when activated with long wave ultra violet light. A blood sample is added to the reaction mixture and after a suitable period of incubation a drop of the mixture is spotted on filter paper, allowed to dry and examined for fluorescence under ultra violet light. By this test, when galactose-1-phosphate uridyl transferase is present NADP is converted to NADPH and fluorescence is detected. If no enzyme is present no conversion takes place and no fluorescence is noted. This principle is also used to detect G-6-P D deficiency. On the other hand, patients with pyruvate kinase deficiency and with glutathione reductase deficiency do not reduce the NADPH already present in the reaction mixture and the fluorescence does not disappear.

BLOOD AND URINE AMINO ACID SCREENING

In 1964, both Efron et al[5] and Scriver et al[6] described the successful use of paper chromatography as a screening test for detection of disorders of amino acid metabolism in blood. In the Efron procedure blood is collected on filter paper while the Scriver procedure uses whole blood collected in heparinized capillary tubes which can be sealed with plasticine. Thus in both cases the blood sample can be mailed to a laboratory where the amino acids can then be separated by descending chromatography. Any individual amino acid present in excess of 6 mg/100ml is ordinarily discernable. Since these levels are usually pathological, this is an ideal method for screening for amino acid disorders.

According to Jackson et al[7] qualitative chromatographic examination of amino acids in urine or blood is the most comprehensive test included in programs for screening for metabolic diseases. After testing the various methods of chromatography, Jackson concluded that the less cumbersome method of thin layer chromatography, which also yields results over a shorter period of time, should be the standard screening method in Toronto's Hospital for Sick Children. The homocystine spot falls in an area in which there is normally a fairly heavy glutamine spot. Since, in homocystinuria, the excretion of homocystine may not be sufficient to intensify this spot beyond the normally observed amount for glutamine alone and since it may not be accompanied by much, if any, increase in methionine, it is possible to miss a case of homocystinuria. Also, cystine stains poorly and significant elevations can be missed. For these reasons, the chromatograms are always supplemented with the cystine nitroprusside test for disulphides. The addition of a simple reducing sugar test makes a combination that will screen for a wide range of metabolic disorders.

Chromatography has proven to be useful not only in the detection of inborn errors of amino acid metabolism but also in following blood concentrations of amino acids during therapy. For example, the patient with Maple-Syrup Urine Disease can be treated at home with a diet low in the branched chain amino acids. A capillary blood sample on a filter paper card can be sent to the laboratory three times a week. If the valine, leucine or isoleucine spots on the chromatogram are weaker or stronger than those of the normal control the physician is notified and an appropriate change is made in the diet. Thus we can conclude that chromatography is one of the most

versatile, efficient, and inexpensive tools which are used in the clinical laboratory for the study of inborn errors of metabolism.

TABLE 3
Some Inborn Errors of Metabolism with Increased Urinary Amino Acids but little or No Increase in Blood Concentration[6]

Disease	*Amino Acids in Excess in Urine*	*Chief Clinical Features*
Cystinuria	"Basic" amino acids, cystine, lysine, arginine, ornithine.	Renal calculi
Hartnup disease	"Neutral" amino acids	Mental retardation Ataxia Pellagra-like rash
Cystinosis	All plasma amino acids	D-resistant rickets Acidosis Dehydration Early death
Joseph's syndrome	Proline Hydroxyproline Glycine	Infantile convulsions Increased C.S.F. protein
Argininosuccinic aciduria	Argininosuccinic acid	Mental retardation Friable hair Ataxia
Cystathionuria	Cystathionine	Mental retardation Congenital abnormalities Psychosis Pituitary disease

SIMPLE URINE TESTS FOR DETECTION OF ERRORS OF METABOLISM[8]

TABLE 4
Some Diseases in which a specific smell has been described:

Disease	*Odour*	*Compound*
Phenylketonuria	Mouse-like	Phenylacetic acid
Maple-syrup urine disease	Maple syrup, sweetish	Branched chain Alpha keto-acids
Cystinuria	Sulphurous	Hydrogen sulphide
Homocystinuria	Sulphurous	Hydrogen sulphide
Isovaleric acidaemia	Sweaty feet, "cheesy"	Isovaleric acid

REDUCING SUBSTANCES

Reagent: Clinitest (Ames).

Method: Five drops of urine are added to ten drops of water in a test tube containing one tablet and the colour is noted after fifteen seconds. (See manufacturer's instructions.)

RESULTS

Reducing Substances Which May Produce a Positive Clinitest:

Glucose — Diabetes mellitus, Fanconi's syndrome.
Galactose — Galastosemia and variants, galactokinase deficiency.
Lactose — Lactase deficiency, congenital or acquired.
Homogentisic acid — Alkaptonuria.
Phenols — Phenylketonuria, tyrosinosis, tyrosine transaminase deficiency.
Fructose — Fructosemia (aldolase deficiency), essential fructosuria.
Xylulose — Pentosuria.
Drugs — Ascorbic acid, chloral hydrate, sulphonamides, P.A.S., tetracycline, and chloramphenicol.

The presence of glucose may be confirmed by the use of any commercially prepared dipstick containing glucose oxidase. More definitive identification of non-glucose reducing compounds requires chromatographic methods.

FERRIC CHLORIDE

Reagent: 10% aqueous ferric chloride.

Method: Add five drops of reagent to one ml of urine. Mix and observe the colour immediately and two to three minutes later.

Results: The colour reaction is not specific. A positive test will result only when the metabolic disturbance is severe enough to cause excessive production of keto acids from corresponding amino acids. The test will detect classical phenylketonuria when the level of plasma phenylalanine exceeds 15 mg/100 ml but will not differentiate the classical form from milder variants of hyperphenylalaninemia which do not require rigid dietary therapy, yet in which the plasma phenylalanine may reach 20 mg/100 ml or more.

TABLE 5
Some Diseases and Compounds Giving a Reaction With Ferric Chloride and/or Phenistix[8]

	Ferric Chloride	*Phenistix*
Phenylketonuria	+ Blue-green	+
Maple-syrup urine disease		
(alpha-ketoisovaleric acid)	+ Blue	+
(alpha-ketoisocaproic acid)	Yellow	+
(alphaketomethyl valeric acid)	Blue-green	+
Homogenistic acid	Transient blue-green	—
Salicylates	Purple	Purple
Phenothiazines	Purple-brown	Purple
Histidinemia	Gray green, stable	Gray

Initial screening of urine to detect metabolic disorders as presented by Berry et al[9] include commercial dip sticks and spot tests. Freshly voided urine specimens are placed in a bottle containing a few crystals of thymol as preservative and refrigerated as soon as possible after collection. A fasting morning sample is usually employed but a random sample may be used. Test strips of Combistix for pH, protein, and glucose and Phenistix for such things as those listed in table 5. Acetest tablets show the presence of ketones and Clinitest tablets indicate the presence of reducing sugars. The urine is also tested Millon's reagent

for tyrosine and parahydroxyphenyl compounds, with 2, 4,-dinitro-phenylhyrazine for keto acids and with anthrone reagent to show the presence of all carbohydrates including non-reducing sugars. Cyanide nitroprusside reagent is used to detect cystine and homocystine, sulphur containing amino acids which have a free sulphydryl group or disulphide bond. A false negative result will occur if the urine is too acidic.

Other specific urine tests include the use of cetyltrimethylammonium bromide to detect excess mucopolysaccharides. Hurler's, Hunter's and Scheie's syndromes consistently show a positive result. The clinical and biochemical abnormalities of these inherited disorders result from excess tissue storage of mucopolysaccharides. Therefore, the test is usually not helpful until after early infancy before which time many false-positives and false-negative results are often obtained. The toluidine blue stain test is simpler and at least as sensitive for the detection of excess mucopolysaccharides.

The o-toluidine test detects the presence of excess copper and the diagnosis of Wilson's disease should be considered. Definitive assay for ceruloplasmin should then be made.

Metachromatic staining of freshly voided urine can indicate the diagnosis of sulphatide lipoidosis (metachromatic leucodystrophy).

The identification of crystals in urine may be difficult, since several compounds may assume different shapes under varying conditions. Uric acid crystals dissolve in an alkaline pH and phosphates dissolve on the addition of dilute acid. Uric acid crystals may occur in normal urine, but large amounts of these crystals suggest the advisability of checking plasma uric acid levels.

No matter how good it may be, a screening test is unlikely to completely eliminate false positive and false negative results. The positive test result may be interpreted in several different ways. It may indicate a variant form of the disease rather than the typical form for which the screening program was established. The variants may require completely different therapeutic approaches. Further studies of any patient with a positive test are always indicated. Constant monitoring of the biochemical and clinical progress of the patient and a high index of suspicion are the best safeguards against failure to recognize an atypical condition, and the induction of a deficiency state by prescription of a diet far below the tolerance of the patient will be avoided. For example, Scriver points out that hyperphenylalanemia may in fact be an index of several conditions, only one of which is the disease phenylketonuria. In summary, the physician should remember that the identification of an aberrant biochemical phenotype is not synonymous with disease, and treatment should not be initiated to restore the normal phenotype until further tests confirm the presence of the disease.

On the other hand, it is inevitable that false negative results will be obtained. For example in phenylketonuria, the plasma phenylalanine level must be greater than 15 mg/100 ml before phenylpyruvate is excreted and the keto acid cannot be synthesized endogenously for several weeks after birth although phenylalanine accumulation begins at birth. Thus early screening for PKU in the neonate may yield a false negative result.

Every infant's urine should be screened, regardless of apparent good health. The testing of urine in the newborn will not rule out some inborn errors of metabolism since the infant has not lived long enough in an extrauterine environment and consumed enough food to accumulate the metabolites which provide diagnostic clues to the underlying enzyme deficiency disease. Ideally, the urine should be tested when the infant is ten days to two weeks of age. Routine testing of infants' urine for the first time at six weeks to two months is too late. By that time irreparable brain damage may have occurred in the infant with phenylketonuria or homocystinuria, and the infant with maple syrup urine disease may have already died of his disease.

SUMMARY

Progress in medicine and technology in recent years has brought the matter of screening for metabolic disorders into prominence. This article presents four approaches to the detection of inborn errors of metabolism in the newborn: 1) bacterial "inhibition assay"; 2) reduction of NADP; 3) use of chromatography; 4) simple and specific urine tests. Physicians must be alert to the opportunities whereby devastating disease can be detected in early life and harm prevented by appropriate therapy.

ACKNOWLEDGEMENT

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Recent Advances

Cystic Fibrosis

Cystic fibrosis (C.F.) is a hereditary disease of children, adolescents and young adults in which there is a widespread dysfunction of exorcrine glands. It is one of the most common inborn errors of metabolism, yet, the basic underlying defect is not understood. In this paper, I intend to review some recent advances in the genetics, diagnosis and pathogenesis of this disease.

GENETICS

Untreated cystic fibrosis of the pancreas is probably the most common lethal genetic disease among Caucasian children. Genetic transmission occurs as an autosomal recessive trait, and there is increasing evidence that a single mutant allele is able to cause the disease. A recent report indicates that the C.F. gene may be localized to the short arm of chromosome #5.[1] The authors have observed a patient with the cri-du-chat syndrome and deletion of the outer part of the short arm of chromosome #5. The child was thus hemizygous for that single set of genes on the homologue of the missing piece. The patient had evidence of cystic fibrosis, an autosomal recessive disease and thereby usually expressed only when both mutant alleles are present in the homozygous state. However, should the mutant gene for C.F. be located in the outer part of the short arm of chromosome #5, this patient who is hemizygous for that segment, could express the disease with only a single mutant gene. (See fig. 1)

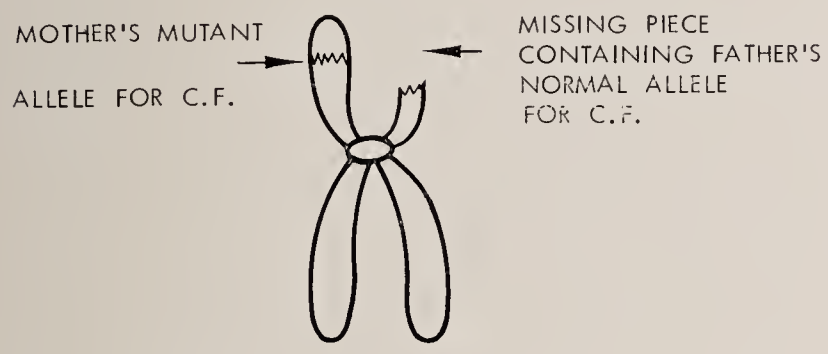

FIG. 1. Chromosome #5 from a portrait with cri-du-chat syndrome and cystic fibrosis.

Since only the mother and not the father had been demonstrated to be a heterozygous carrier for C.F. (by the Spock test (see below) it can be concluded that the father's normal allele must be on the missing piece of chromosome #5. This evidence favours localization of the C.F. gene to the outer one-third of the short arm of chromosome #5.

DIAGNOSIS

The major clinical features of cystic fibrosis are as follows:

1) chronic pulmonary disease
2) pancreatic insufficiency
3) high concentration of sweat chloride

Other clinical features which may be seen include the following:

4) nasal polyps
5) chronic sinusitis
6) rectal prolapse

A definitive diagnosis of C.F. depends not only on the history and physical findings, but also requires at least two of the following:

1) abnormal radiograph of the chest
2) abnormal sweat chloride test (chloride concentration $>$ 60 meq/L.)
3) abnormal pancreatic function tests

Recently, three new tests have been proposed for diagnostic use:

1) Kopeto et al[2] reported increased sodium concentrations in the nails of patients with C.F. Potassium levels were also raised, but the overlap with normals was too large to be of practical use.

2) Danes and Bearn[3] have reported the presence of a genetic cell marker in C.F. Skin fibroblast cultures derived from seven children affected with C.F. have shown a unique and easily recognizable cytoplasmic intravesicular metachromasia. Thirteen heterozygotes for C.F. (parents of patients) were also investigated and in all instances a similar cytoplasmic metachromasia was seen. Normals have shown only an occasional metachromatic cell.

3) Spock et al[4] detected an euglobin factor in the serum of patients with C.F. which causes a disorganized rhythm of cilial movemet in cultured explants of rabbit respiratory tract epithelium. (See fig. 2)

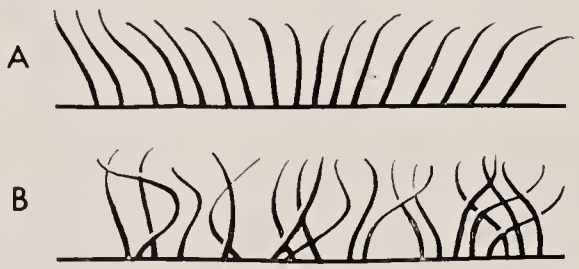

FIG. 2. Diagramatic representation of rabbit respiratory epithelium. A. Normal ciliary beat; B. Disorganized beat produced by serum of cystic fibrosis patients.

This "Spock test" is consistently positive using sera of patients with C.F. and has been negative using sera of patients with a variety of diseases with clinical features similar to those seen in C.F. Most important, the parents (heterozygous for the C.F. gene) gave consistently positive results if their sera were concentrated 2.5 times.

The significance of the discoveries of the cytoplasmic metachromasia and the Spock test lies in the fact that these tests may permit recognition of the heterozygous state for C.F. and thus facilitate studies in genetic linkage and provide an opportunity for genetic counselling.

The presence of cytoplasmic metachromasia offers an experimental opportunity to study the primary defect of this commonly inherited disease at the molecular level.

PATHOGENESIS

The common denominator responsible for the widespread dysfunction of exocrine glands and therefore for the pathogenesis of C.F. has thus far escaped detection. It is generally accepted that an abnormal physicochemical behaviour and an abnormal chemical structure of mucus probably play major roles in initiating the chain of clinical events leading to many of the symptoms manifested by patients. These abnormalities of mucus secretion result in obstruction of pancreatic ducts, bile canaliculi, intestinal glands, salivary ducts and glands, paranasal sinuses, respiratory bronchi and seminiferous tubules.

This classical theory has recently been modified by Johansen et al[5]. Histological examination of mucous glands consistently reveals mucous cells swollen and distended with their secretory products. No abnormal mucosubstances can be demonstrated in any specified type of mucous cell by histochemical criteria. These observations suggest that intracellular mucoid material may simply be more concentrated than normal. It may be that the synthesis of mucoid secretory products does *not* follow an abnormal pathway at all, but that the newly synthesized material may be insufficiently diluted during elaboration of the final secretion within the cell. This insufficient dilution, the authors speculate may be due to inhibition of fluid movement across the cell from the extracellular space. Secretory products accumulate within the cell because it is more difficult to extrude the thickened material to the exterior if it cannot be sufficiently diluted. The event which initiates the pancreatic changes in C.F. may well be the lack of or decreased secretion of the secretin-stimulated bicarbonate-rich, watery electrolyte fraction of pancreatic juice, owing to the inhibition of fluid movement across the epithelial secretory cells. This results in a highly concentrated final secretion which is probably directly responsible for the actual tissue damage or destruction. This mechanism is thought to be operative in the elaboration of bile in the liver as well as the elaboration of mucus in the lungs and gastrointestinal tract.

The authors further speculate that such a mechanism also exists in the secretions of serous glands (sweat and salivary glands) as will be discussed below.

Important constituents of the extracellular space of connective tissues are the acid mucopolysaccharides (AMPS). These macro-molecules bind cations and play a role in maintaining water and electrolyte balance. These AMPS have a short half-life and are affected by changing levels of circulating hormones.

An amorphous layer has been demonstrated external to the triple layered plasma-membrane of many secretory cells which is believed to be mucoid or polysaccharide in character. This extraneous coat is synthesized by the underlying cell. Its synthesis may be influenced by circulating hormones, as is the case with AMPS. It is important to realize that the mucoid coat consists of complex polyelectrolytes and therefore can be expected to affect the movement of fluid and electrolytes to and from the cells if there is a disturbance of circulating hormones.

When exocrine cells are stimulated there is a change in membrance potential and this may affect the mucoid coat so that the movement of water and charged molecules through the cell plasma membrane itself may be modified. Circulating hormones may also react with the AMPS or the mucoid coat thus modifying the movement of water and electrolytes up to the cell membrane.

The authors put forth the possibility that the inhibition of fluid movement into secretory cells may be related to an abnormality in the extracellular mucosubstances described above. In C.F. patients this abnormality may be due to an imbalance or disturbance of the hormones controlling the biosynthesis of the mucopolysaccharides associated with the exocrine tissues. In the sweat glands it is postulated that the abnormal mucoid coat lies on the luminal surface of the tubule cells. This coat prevents the movement of chloride from the sweat into the gland thus giving rise to the high sweat chloride values.

The abnormality may also be due to competitive inhibition of the normal humoral factors by a circulating polar polypeptide as suggested by Mangos et al.[6]

A summary of the hypothesis for the pathogenesis of C.F. is as follows:

1) There is an inhibition, imbalance or disturbance of circulating hormones that control the synthesis of extracellular mucosubstances or the mucoid coat.

2) The abnormal extracellular mucosubstances inhibit the movement of water and electrolytes into the secretory cells.

3) As a consequence, secretory products are insufficiently diluted and accumulate within the cell.

4) When the thickened secretions enter the lumen, they give rise to the classical obstructive picture.

It should be pointed out that this hypothesis represents only one possible explanation for the pathophysiology of cystic fibrosis. There is as yet no proof that hormonal factors play such a role as outlined above. The interested reader is referred to an excellent review of cystic fibrosis by di Sant'Agnese[7] where many other areas not considered in this paper are examined.

Paul Spring 7T0

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Assaeries

A New Metabolic Disease

It is only in recent years that medical science has begun the long process of interpreting the pathogenesis of disease in biochemical terms. Not infrequently these elaborate and painstaking investigations have led to the unexpected reward of an exciting accidental discovery. We are pleased to announce that once again a new metabolic disease has been uncovered unwittingly in the laboratory, revealing a disorder which previously had been not only gravely misunderstood but also completely unsuspected. To date we have positively identified seven cases, but we feel this may prove to be of great significance, insofar as our study does not yet include Australia or the Communist countries of Eastern Europe. We have named the disease Malu Yud, after the deceased father of our first flask-washer. The remainder of this article attempts to summarize the clinical and biochemical phenomena which characterize the disease.

CLINICAL SYNDROME

Malu Yud is characterized, in general, by a variety of seemingly unrelated signs and symptoms. These include coarse hair on the arms and legs, webbing of the feet proximal to the base of the toes, whorl and/or loop patterns in the fingerprints, and dietary intolerance to ipecac. In addition, the patient may or may not exhibit chills and/or sweating when exposed to heat or cold; he usually has blue eyes, or brown or green, bilateral zamboni reflexes on each side, and an elevated serum oregano; and may on occasion be found to have an aberrant anus, though no such cases have been reported yet.

One must keep in mind that the above description is in no sense meant to clarify the condition, for almost any or all of the aforementioned signs may or may not be present or absent in any one particular case or another, all things being equal.

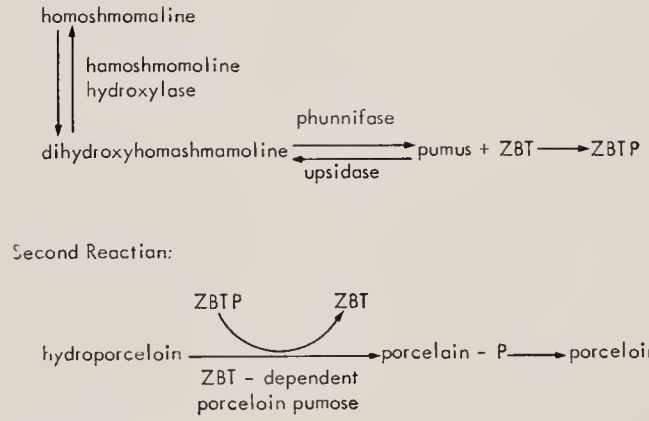
THE BIOCHEMICAL DEFECT

It is in the laboratory that Malu Yud was first encountered, and it is in the area of biochemistry that the disease is most clearly understood. Initial studies were performed on samples of saliva carefully extracted by suction curette from the ducts of Rivinus, and slowly simmered at 62°C for 37 minutes at sea level in a Turkish bath. Abnormal coagulation was observed by accident when the specimens were mistakenly left out overnight on a penicillin mold.

The subsequent twenty-six years of study have revealed a whole new enzyme system common to almost every tissue in the human body.

The critical enzyme in the system is phunnifase, which catalyzes the conversion of dihydroxyhomoshmomoline to pumus. In the normal individual pumus is subsequently incorporated into almost all energy processes in that it reacts with the coenzyme ZBT in the oxidation of porcelain. This reaction is of prime importance in the central nervous system, where pumus is now understood to form the basis of most human thought processes. However, the defect is in none of the aforementioned reactions, which all function normally in the patient afflicted with Malu Yud. The defect is in the presence of an excess of the enzyme which is the reverse catalyst to phunnifase, that enzyme of course being upsidase (as distinct from upsidase — E). The excess of upsidase, now known to be due to the absence of as yet unidentified inhibitors which normally augment the effects of the repressor substance that controls the operational genes governing the production of factors which influence the genes that manufacture upsidase, leads to an excess of homoshmomoline, which accumulates in the tissues. It is not yet clear if the clinical manifestations are the results of excess homoshmomoline, or a supply of pumus inadequate for normal growth. It must be realized that while pumus can be ingested in the diet, it is not absorbed, but passes through the alimentary canal virtually unchanged, appearing at the anus in its usual familiar form.

The biochemical reactions are summarized diagrammatically below.



DISCUSSION

It must be recognized that the prognosis and long-term management of Malu Yud are mysteries yet to be investigated thoroughly. Nevertheless there can be no question of the value of the disease as an exemplary educational tool for the students of biochemistry and of clinical precision. It is our sincerest hope that medical educators will not allow so useful a gem to pass another year without a prominent and significant place in their teaching programs.

A. J. Shoichet

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Special acknowledgement to Larry Yud, with and/or without whom.

The concept of body temperature and its regulation is indeed old. Sanctorius Sanctorii, in 1614, recognised skillfully that coitus produced variations in body temperature. Thus, the conclusion at that time was that body temperature fluctuations did occur under physiological conditions. Ever since that time, the mechanisms of accurate body temperature control have been the source of much research and bewilderment.

HEAT PRODUCTION

The homeostatic mechanisms within our bodies keep body temperatures between narrow limits by means of a balance between heat production and heat loss. The human body gains heat through:

(a) Basal metabolism and physical activity. An average adult male can produce between 70 to 550 calories/M^2/hr.

(b) Involuntary muscle activity as in shivering will also increase heat production.

(c) Certain foodstuffs have a specific dynamic action and thus affect heat production. Protein may cause as much as a 20% increase in heat production.

(d) In some instances heat may be gained from the environment.

HEAT LOSS

Eighty five percent of the total heat produced is lost through the skin by conduction, convection, radiation and evaporation. The lungs account for the loss of 14% through evaporation, warming of expired air and removal of CO_2 from solution.[2] The remaining small portion is lost in the urine and feces.

In the comfortable zone of external temperature (28°C - 31°C), about 65% of the total resting heat production is lost through radiation (loss of heat from a warm object to a relatively cooler object not in contact with it), conduction (loss of heat from a warm to a cool object in contact with it), and convection (loss of heat through air currents). As the atmospheric temperature rises above the comfortable zone, less heat is lost by radiation and convection and when atmospheric temperature equals that of the skin, no heat is lost by these mechanisms.

Evaporation is another important mechanism in keeping the internal environment constant. As external temperature and/or physical activity increase, sweating and evaporation will occur. Each gram of water, deprives the body of 0.58 cal. when it evaporates from skin through insensible perspiration or sweating, or from lungs through increased respirations. Heat loss by evaporation depends upon the surface area and the humidity of the environment. We are thus uncomfortable on hot humid days because the atmosphere is saturated with water and evaporation cannot occur effectively to distribute heat.

THE TEMPERATURE REGULATING CENTER

The 'temperature eye', as elucidated by Benzinger and his colleagues in 1961, is in the hypothalamus, situated at the floor of the third ventricle of the brain. According to current views, the hypothalamus contains two types of neurons. While these cells have not been histologically differentiated, experimental evidence points out that one cell type responds to an elevation of the temperature of the blood perfusing the hypothalamus while the other cell type responds to a depression of the temperature of the blood. The anterior part of the hypothalamus contains the neurons susceptible to a rising blood temperature. Their descending pathways extend down the brain stem close to the central grey matter and set up connections with respiratory and cardiovascular mechanisms of the brain stem and spinal cord. Thus when blood temperature is elevated, the discharge evoked from these neurons set into play the peripheral processes which contribute to heat loss, i.e. respiratory changes, peripheral blood flow changes, and perspiration.

The neurons affected by falling blood temperature are located in the hypothalamus mainly below the supraoptic region. These neurons have descending pathways similar to those previously mentioned. When these neurons discharge, the process of elevating body temperature is set into motion. This process includes increased production of heat within the body as well as conservation of heat by reducing radiation.

PHYSIOLOGICAL EFFECT OF COLD

On exposure to cold, the hypothalamus adjusts the heat balance by:

(1) Preventing excessive heat loss. Impulses are fired to sympathetic vasomotor centres in the medulla and spinal cord. This leads to constriction of the arteriovenous anastomoses of the skin. The blood flow through the skin is thus reduced and the skin temperature drops. In this way, there is increased insulation thus minimizing the loss of heat by convection, conduction and radiation.

The positioning of the body is also important in preventing heat loss. Curling up (fetal position) allows one to decrease the body surface area exposed to the environment and in in this way decreases heat loss.

(2) Increasing heat production.

(a) Shivering. The neural patterns responsible for shivering are not well understood, but may be mediated by extrapyramidal fibers. Often accompanying shivering is piloerection or 'goose-bumps'. This tends to increase and aids the vasconstrictor impulses in reducing cutaneous blood flow.

(b) Adrenaline and nor-adrenaline secretion are stimulated due to the stress of the cold environment. This increases the metabolic rate, raises blood glucose levels and aids the vasoconstrictor impulses in reducing cutaneous blood flow.

(c) On exposure to cold, there is hypothalamic activity which results in a general metabolic speed-up. Thus there is increased release of the thyrotropic hormone with resulting increases in serum thyroxine levels which further potentiate the raised metabolic rate.

(d) Adrenal cortical hormone secretion is also increased due to increased hypothalamic and pituitary activity causing an increase in ACTH release.

PHYSIOLOGICAL EFFECT OF HEAT

On exposure to heat, the hypothalamus adjusts the heat balance by dissipation of heat through the following mechanisms:

(1) Cutaneous vasodilation and increase in the blood volume. The cutaneous arterioles, capillaries and arteriovenous anastomoses are reflexly dilated.[2] The increase in blood volume is due partly to contraction of the spleen and partly caused by the withdrawal of fluid from the deeper tissues. The cutaneous blood flow is considerably increased. The skin temperature rises and the heat loss by convection and radiation are facilitated despite the high external temperature.

(2) As environmental temperature rises, the avenues of heat loss become progressively restricted until atmospheric temperature equals skin surface temperature at which point sweat secretion, and evaporation are the only methods remaining for heat loss from the surface. In this situation evaporation of sweat accounts for all the heat loss and thus must be equal to total heat production so that the average body temperature will remain constant. At still higher temperatures, the loss of heat through evaporation of sweat must equal all the heat produced by metabolic processes plus the heat gained from the atmosphere by convection and radiation. As much as two litres of sweat may be secreted per hour when severe exercise is performed. If all of it evaporates from the surface of the body, it would account for the loss of 200x0.6=1,200 calories,[2] which is double the maximum heat production in prolonged effort.

DISORDERED THERMOREGULATION (FEVER)

Fever, simply defined is an elevation above the normal body temperature of 98.6°F plus or minus the daily fluctuations in temperature. Fever upsets the 'temperature eye' and adjusts the thermoregulatory mechanism to maintain body temperature at a higher than normal level. All fevers are accompanied by an increase in heat production. Each 1°F rise in body temperature increases metabolic activity by 7 - 10%. Furthermore, there is a decrease in heat loss accompanying this fever due to the fact that the disordered thermoregulatory center has been 'set' at a higher than normal level.

PATHOGENESIS OF FEVER

In spite of our precise knowledge concerning thermoregulation as yet relatively little is known about pathogenesis of fever; i.e. the way in which disease upsets thermoregulation. In 1948 Beeson reported the presence of an endogenous pyrogen in rabbit polymorphonuclear leukocytes. At that time, his report was discarded. Today, the granulocytic factor that he discovered is believed to have a central role in the pathogenesis of fever. The granulocytic pyrogen was first demonstrated in the study of experimental fevers produced in rabbits by the intravenous injection of bacterial endotoxins. This endotoxin gives rise to the production of an endogenous pyrogen whose plasma concentrations correlate well with the severity of the fever and whose properties are indistinguishable from those of granulocytic pyrogen. Thus, the endogenous pyrogen seems to act directly on the hypothalamus and is derived from the rabbit's granulocytes.[5] Moreover it has been possible to produce endogenous pyrogen in vitro, by the interaction of normal granulocytes with the injected agent (endotoxin) in normal serum at 37°C. On introduction of the organisms into normal serum, phagocytosis is the initial response. Then these granulocytes release pyrogen, indicating that the release of pyrogen is somehow triggered by phagocytosis. When phagocytosis is inhibited chemically no pyrogen is released. Thus endogenous pyrogen derived from polymorphonuclear leukocytes, and possibly involving their lysosomes, appears to be the trigger molecule necessary for the production of many experimental fevers. There is also reason to believe at present that this 'trigger' molecule plays a similar role in many human fevers.

The above experimental evidence does not account for fevers in patients with agranulocytosis or other diseases in which mononuclear cells rather than granulocytes dominate the pathologic lesions. Experimentally, monocytic suspensions prepared from bloods of normal human subjects and patients with agranulocytosis and monocytic leukemia were found to release endogenous pyrogen after phagocytosis of heat-killed staphylococci.

In summary, the experimental findings indicate that mononuclear cells as well as granulocytes are capable of generating endogenous pyrogen. This capability provides a convincing explanation for the fever of diseases in which granulocytes are not prominent in the pathologic lesions.[5]

DIFFERENTIAL DIAGNOSIS OF FEVER

1. Acute Febrile Illness

   (a) Infectious
   — viral
   — bacterial

   (a) Allergic
   — drug reactions
   — incompatible blood reactions

   (c) CVS
   — thromboembolic disease
   — hemolytic crises
   — septicemia

   (d) Rheumatic disease
   — gout
   — rheumatic fever

2. Prolonged Febrile Illness

   (a) Infectious — bacterial
   — tuberculosis
   — endocarditis
   — brucellosis
   — salmonella
   — osteomyelitis
   — abscess
   — cholangitis
   — bronchiectasis
   fungal
   — histoplasmosis
   — coccidioidomycosis

   (b) Collagen disease
   — lupus erythematosus
   — periarteritis nodosa
   — rheumatoid arthritis
   — rheumatic fever
   — Henoch — Schonlein purpura

   (c) Neoplastic disease
   — sarcomas — bone and lymphoid tissue
   — carcinoma — stomach
   — pancreas
   — Hodgkin's disease

   (d) Hematopoietic disease
   — leukemias
   — hemolytic episodes

   (e) CVS
   — myocardial infarct
   — congestive heart failure
   — arteritis
   — pulmonary embolism and infarction

   (f) Iatrogenic — drug eruptions

   (g) Dermatologic
   — ichthyosis
   — congenital absence of sweat glands

FEVER OF UNKNOWN ORIGIN (F.U.O.)

F.U.O. presents a challenging diagnostic situation. There is a wide variety of possible causes of F.U.O. (outlined above). The clinical differentiation of these conditions is often difficult and sometimes even impossible. This is because many of the above disorders manifest themselves in identical ways. Thus, chronic infections, collagen-vascular disease, new growths, drug reactions, leukemias and granulomas, which collectively are among the most common cause of F.U.O. may all be associated with chills and sweats, weight loss, dermatologic disorders, arthritis, muscle weakness, neuritis, hepatosplenomegaly, lymphadenopathy, blood dyscrasias, and alteration in the serum protein. Conversely for varying periods of time the only clinical sign may be fever with its accompanying symptoms as general malaise, headaches, weakness, anorexia and irritability.

In making a diagnosis of F.U.O. an accurate, complete history and physical examination is of course necessary. However, the scope of this brief article does not allow for a detailed analysis of the above. We would rather concentrate our efforts on areas of the physical examination which are often neglected but which may provide important diagnostic signs in F.U.O.

1. Eye Grounds

Many systemic disorders express themselves by alterations in or about the eyes. Episcleritis with limbal infiltrates producing a reddened, weeping eye is often associated with polyarteritis nodosa. This condition can subsequently be substantiated with a muscle biopsy. Choroiditis may be part of a miliary tuberculosis. The so-called 'cytoid bodies' are seen in 15% of patients with S.L.E. The sole indication of sarcoid may be a nodule in the iris. A brilliant conjunctivitis may suggest S.L.E., polyarteritis, or drug reaction.

2. Oral Cavity

Swollen, sore, boggy, bleeding gums may indicate leukemia, or dilantin usage. A dirty mouth with dental caries and infected gums may be the nidus of infection for bacterial endocarditis, pneumonitis, bronchiectasis or lung abscess. Petechiae, splinter hemorrhage and Roth spots are not specific for bacterial endocarditis as they represent a local area of vasculitis and hence may be seen in S.L.E., sepsis with endocarditis, rheumatic fever, drug reaction or polyarteritis. Ulcerated lesions in the oropharynx have a multitude of causes; however, syphilis, T.B. and histoplasmosis are sometimes overlooked.

3. Lymph Glands

Any malignant tumor may present with F.U.O. and as such may make its clinical appearance as small lymph nodes about the clavicles and deep in the axillae. Thus exceedingly careful palpation is necessary. Granulomatous lesions as well as sarcoid may involve the small glands in this area which are readily accessible to biopsy.

4. Diaphragm

Movement of the diaphragm will be inhibited by an inflammatory process affecting it. A hemidiaphragm on one side which fails to move freely and shoulder tip tenderness on the same side may indicate a subdiaphragmatic abscess.

5. Auscultation of Head, Liver, Abdomen, and Tender, Painful Areas

Vascular tumors such as sarcomas and hypernephromas may produce a bruit. A.V. malformations and temporal arteritis give rise to bruits. The presence of a liver abscess is often difficult to establish clinically. One should listen over the surface of the liver in an effort to hear the friction rub which may accompany this process.

6. Rectal Examination

It should not be necessary to emphasize the need for this procedure. Yet, it is sometimes omitted. One is particularly interested in any signs of perirectal infection, neoplasm in the rectum or pelvis and infection in the prostrate and seminal vesicles. If a perirectal abscess is present, one must search for the underlying cause such as leukemia and other disorders with a decreased immune response, such as Crohns' disease and granulomas of the intestine.

7. Testicles

The testicles may be the sight of tumors which may introduce themselves with fever. In young men these may metastasize to the mediastinum and a diagnosis of lymphoma may be made because the testes have not been palpated. Tenderness or atrophy of the testes may point to a diagnosis of polyarteritis nodosa. This is subsequently proved testicular biopsy. Granulomatous disease of the testes particularly in older patients should not be overlooked.

8. Temperature Chart

It is helpful to study the course of a patient's temperature. Has the fever been constant or recurrent? Hodgkin's disease is one of the more common causes of intermittent fever with relatively good health in the intervals. Bronchiectasis, recurrent pulmonary emboli, pericarditis, S.L.E., urinary tract infection, ulcerative colitis, and drug reactions may also result in intermittent fever. The height of the fever is usually not particularly helpful in the diagnosis.

Spiking temperatures are usually indicative of a septic process but are also associated with S.L.E., drug reactions, lymphomas, and hypernephromas. A fever which spikes twice every 24 hours is characteristic of gonococcal endocarditis.

Laboratory and x-ray techniques are also invaluable in establishing a diagnosis. However, they will not be expanded upon at the present time.

CLINICAL STATISTICS[6]

Taken from a series of 347 patients

Table 7 — *Patients with fever for more than 2 weeks*

Diagnosis established within 2 weeks	287 (83%)
Diagnosis after 2 weeks	33 (9.5%)
Diagnosis remaining unknown	27 (7.8%)
Total admissions to hospital	5182
Proportion of fever cases	6.6%

Table 2 — *Diagnosed within 2 weeks of fever*

No. of cases	287
Infections (mainly pyogenic and T.B.)	65%
Neoplastic disease (leukemia, carcinoma, lymphoma)	10%
Collagen diseases	8%
Liver disease	3%
Miscellaneous	14%

Table 3 — *Fever for greater than 2 weeks (17.3% of cases)*

	No. of cases
A. Infectious disease	
T.B.	5
Staph septicemia	1
Mononucleosis	1
B. Neoplastic	
Ca of lung	5
Ca of pancreas, liver or gallbladder	6
Hodgkin's disease	3
Ca of colon	2
Ca of ovary	1
Ca of kidney	1
Sarcoma	1
C. Miscellaneous	
Sarcoidosis	2
Temporal Arteritis	2
Allergic	1
Ulcerative colitis	1
Cholecystitis	1
D. Fever remaining unknown	27

SUMMARY

The mechanisms of temperature regulation were presented from a physiological viewpoint. An effort was then made to demonstrate how thermoregulation was altered in disease states by producing fever. Some basics in physical diagnosis were then outlined. The workup in diagnosis of F.U.O. as presented in this article was by no means complete. It was mainly to acquaint the reader with the more common disease producing fever. It is indeed the hope of the authors that the reader will be stimulated sufficiently to delve further into the topic of 'fever', diagnoses and treatment.

Z. Cohen, T. F. Shapero

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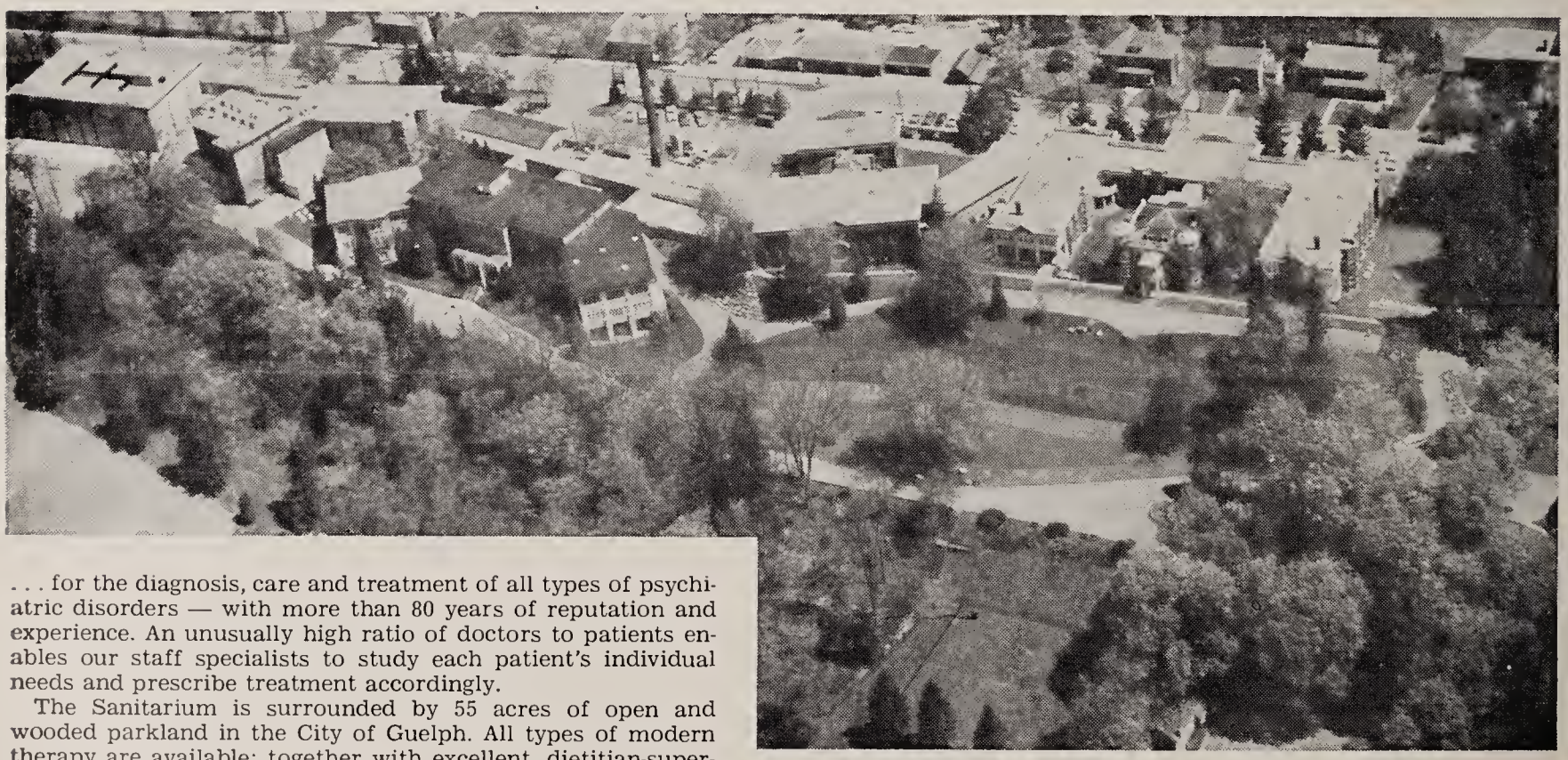

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Persistent Jaundice And Failure To Thrive In A Male Infant

CASE PRESENTATION

A six and one half week old male infant was admitted to hospital because of failure to thrive, listlessness and drowziness.

He had been the product of a full term normal pregnancy, and an uneventful delivery, and had appeared normal at birth weighing ten pounds, three ounces. He was discharged on the fifth day, having had frequent liquid green stools. These gradually improved and became yellow and formed by the 10th day of life. On the 12th day, his mother noticed him to be slightly jaundiced. This deepened over the next three days and then improved although it did not disappear completely. His urine at this time was tea coloured. During the height of the jaundice and continuing for two weeks thereafter he vomited bile stained feedings, and diarrhea began again. There was no hematemesis. He had been breast fed since birth. Suspecting intolerance to this milk he was put on 2% Lactic Acid Milk (LAM). His stools returned to normal and the vomiting ceased, although no weight gain was noticed. Because he continued to lose weight, was unsatisfied and colicky, his formula was again changed, this time to 2% Evaporated Milk, on the 22nd day of life. He started to gain weight. However on day 40, the jaundice deepened and once again he had bright yellow formed stools and tea coloured urine. He continued to take his feedings well. Constipation developed and on day 43 he lost interest in feeding. Two days later he refused all but 4 ounces of his feedings, became listless and was subsequently admitted to the Hospital for Sick Children in Toronto.

The patient did not have any siblings and his parents were not consanguenous and were in excellent health all their lives.

On admission, physical examination revealed a poorly nourished six and one half week old male infant, who was pale, jaundiced, and weak. His temperature was 99.8°F. per rectum, heart rate 100 beats/minute, respiratory rate 40/minute and weight 8.4 pounds. His skin was an ashen yellow colour with fair turgor; there was no subcutaneous fat. His respirations were noisy with course breath sounds and referred rhonchi from the upper respiratory tract. The cardiovascular system was within normal limits. The abdomen was slightly distended but no tender areas or ascites were demonstrable. The liver was palpable 4.5 cm and the spleen 3.5 cm below their respective costal margins. There was no lymphadenopathy, but generalized wasting of muscles was present. He was listless but responded to stimuli and moved all limbs actively. The Moro reflex was intact. The anterior fontanelle admitted three fingers. His sclerae were moderately icteric and slightly injected. His eyes were clear to fundoscopy and reacted equally to light and accommodation.

Urinalysis revealed 3+ albumin, 3+ reducing sugar, no acetone, 1+ bilirubin and 1+ urobilin, with many granular casts on microscopy. The hemoglobin was 8.8 gm%. The blood smear showed hypochromic microcytic anemia with many target cells; there were some atypical lymphocytes and normally appearing platelets. Osmotic fragility testing of the erythrocytes was normal. Blood cultures were negative. The patient's blood was group O Rh— and Coomb's test negative, while his mother's blood was group A Rh+. Liver function studies included a total serum bilirubin of 11.1 mg% with the direct reacting being 3.65%, a cephalin cholesterol flocculation test of 3+, a thymol turbidity test of 4.6 units and a prothrombin time of 16 seconds. Total serum proteins were 6.04 gm% (albumin 3.59%, globulin 2.45% and the gamma globulin fraction was 1.28%). Both the patient and his mother were Wasserman negative.

On the fourth hospital day the reducing sugar in the urine was identified as galactose. A chromatogram of the urine for amino acids was abnormal at this time, there being a high concentration of neutral amino acids. His feeding formula was immediately changed to Mulsoy. Subsequent analysis revealed no galactose, albumin or amino acids in the urine.

His course after the diet change was unaltered. His weight and temperature fluctuated between 8.8 and 9.4 pounds and 97° to 99°F. per rectum respectively. On the 17th hospital day he vomited twice some dark fluid with mucus, for which vitamin K and blood transfusions were given. The patient never rallied and expired twenty days after admission.

DIFFERENTIAL DIAGNOSIS

The most prominent features of this patient's disease on admission were 1) a regurgitation type of jaundice, 2) gastrointestinal disturbances and 3) failure to thrive. The differential diagnosis must consider the many conditions which give this picture at this age.

Assuming that the three aspects are related to one pathological process, the most significant finding is that

of a persistent jaundice: the gastrointestinal complaints and failure to thrive can then be explained secondarily to any of the diseases producing jaundice. It should be noted that this is not a physiological jaundice, as it developed after the 12th post-natal day in a full term infant.

Disease entities which should be considered in the differential diagnosis of regurgitation jaundice in infants were considered and excluded in this child. Congenital biliary atresia usually develops at two to three weeks of age, but the jaundice becomes a deep olive green. This plus the fact that the stools were always coloured and urobilin present in the urine, make this an unlikely diagnosis. The fact that there was a normal osmotic fragility of red blood cells and no evidence for a hemolytic disease, the Coomb's tests being negative, excluded the possibilities of congenital hemolytic anemia and erythroblastosis fetalis. Sepsis was ruled out by urinalysis and a negative blood culture, and the fact that the jaundice was of a gradual rather than a sudden onset. Syphilis was highly unlikely as both the patient and his mother were Wasserman negative. His mother's health was excellent throughout the pregnancy, minimizing the possibility of the rubella syndrome. Intrahepatic obstructive jaundice (cholestasis) due to drug or viral agents, etc., was unlikely in the absence of a history of drug ingestion or signs of complete biliary obstruction. Cytomegalic inclusion disease, toxoplasmosis, herpes simplex, fibrocystic disease of the pancreas and microcystic disease of the liver and kidneys were all considered in the differential diagnosis as well as "neonatal hepatitis", but the conclusive diagnosis of galactosemia and the response to therapy made the detailed investigation in these directions superfluous.

The findings of abnormal liver function tests in relation to parenchymal function of the liver (e.g. abnormal flocculation tests, increased gamma globulin levels, elevated prothrombin time) are quite regularly found in galactosemia while their occurrence in the conditions discussed previously is very infrequent in this age group.

For completeness, the Dubin-Johnson syndrome, an autosomal dominant benign condition, is usually first noticed in adult life thus excluding it in this situation. Other causes of jaundice which appear in this age group (congenital spherocytosis, glucose-6-phosphate dehydrogenase deficiency, vitamin K overdose, Gilbert's disease, Crigler-Najjar syndrome, breast milk containing pregnane-3-alpha 20-beta-diol, cretinism and pyloric stenosis), produce a retention type of jaundice with bilirubin hardly ever occurring in the urine; thereby making the possibility of their presence in this patient very unlikely.

AUTOPSY FINDINGS

Gross Examination

The subject was an extremely emaciated and underdeveloped male of nine and one half weeks, with a loose large abdomen, pale thin skin with the presence of some petechial hemorrhages, and no subcutaneous fat palpable. The body length was 21 inches, body weight 9.0 pounds and head circumference 15 inches all being well below the 10th percentile for a nine and one half week old male infant. The anterior fontanelle was soft and measured ¾ by 1 inch. No opacities were noted in the lens.

The peritoneal cavity contained 100 cc of a turbid, yellow-tinged fluid which grew E. coli. An organizing, purulent exudate was found under the diaphragm and in the upper abdomen. The liver weighed 154 gm (normal 136 gm), was firm but not nodular, and the entire surface was covered with the purulent material. The external as well as the cut surface were dark green and smooth. There was no increase in lobulation markings, and the extrahepatic bile ducts were patent.

The spleen was greatly enlarged at 46 gm (normal 13 gm) and similarly covered with pus. It was congested and the cut surface was smooth and diffusely dull red. There were no esophageal varices but the lower two-thirds was congested and a small ulcer was present at the lower end.

Microscopic Examination

The liver exhibited severe diffuse fibrosis, infiltrating around every hepatic cell cord (pericellular cirrhosis), while the liver cells themselves showed a very marked atrophy and slight fatty change. There was a small amount of inspissated bile pigment seen, as well as considerable bile duct proliferation in the portal areas with no evidence of any inflammatory infiltrate.

The small ulcer on the lower end of the esophagus showed no evidence of hemorrhage and was heavily infiltrated with polymorphonuclear leukocytes. There was a marked omphalitis. The skin showed a small number of petechial hemorrhages. The basal ganglia and parietal cortex were normal although some nuclei appeared to be dying.

All other systems were within normal limits both grossly and microscopically.

The clinical as well as the pathological findings prove the diagnosis of galactosemia conclusively.

DISCUSSION

Galactosemia is an inborn error of metabolism involving a single autosomal recessive gene, which codes for the enzyme galactose-1-phosphate uridyl transferase, necessary for the metabolism of galactose. If this enzyme is absent, in the presence of galactose (ingested in the form of milk lactose) the clinical manifestations of galactosemia result; thus by itself this enzyme defect is harmless but, if galactose is present, incomplete metabolism results and toxic by-products, as yet unidentified, accumulate.

The metabolism of galactose is as follows:

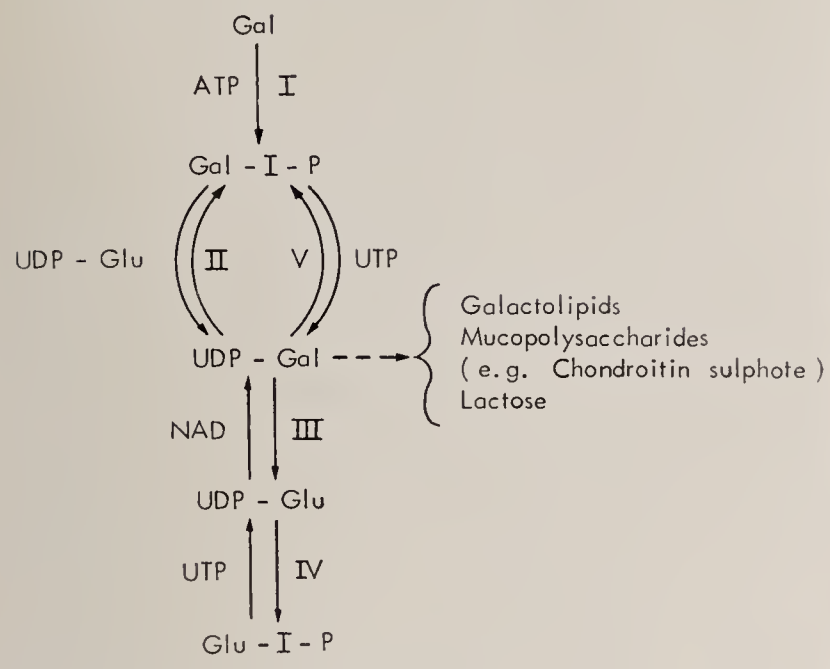

FIG. 1. The Metabolism of galactose.

ABBREVIATIONS:
I — Galactokinose (in brain, liver and erythrocytes)
II — Galactose-1-phosphate Uridyl Transferose (absent in Galactosemia)
III — Uridine Diphosphate Golactose-4-Epimerase
IV — Uridine Diphosphate Galactose Pyrophosphorylase (liver, erythrocytes, broin mommory gland ond muscle)
V — Uridine Diphosphate Goloctose Pyrophosphorylase
Gal — Galactose
Gal-1-P — Galactose-1-phosphate
UDP-Gal — Uridine Diphosphate Goloctose
UDP-Glu — Uridine Diphosphote Glucose
Glu-1-P — Glucose-1-phosphate
ATP — Adenosine Triphosphote
UTP — Uridine Triphosphate
NAD — Nicotinamide Adenine Dinucleotide

Kalkar et al were the first to show the congenital absence of galactose-1-phosphate uridyl transferase. UDP-Galactose is required in forming tissue glycolipids and lactose in adult females. This can be accomplished in galactosemia because enzymes III and IV catalyze reversible reactions. Enzyme V is also effective for this purpose in adults but its activity is much lower in the neonatal period.

In the classic form of galactosemia the infant will present with a history of being normal at birth and 3 - 4 weeks later of developing failure to thrive, vomiting, diarrhea and hepatosplenomegaly. Subcutaneous bleeding may take place. Jaundice is nearly always present and is of the regurgitation type, both the direct and indirect serum bilirubin being elevated. Cataract formation (zonular or lamellar), loss of subcutaneous fatty tissue, and mental retardation are usually a later manifestation of the untreated disease.

Laboratory examination reveals elevated serum galactose and bilirubin levels, abnormal galactose tolerance curve, deficiency of Gal-1-P uridyl transferase in erythrocytes, galactosuria, amino aciduria (mostly the neutral type), albuminuria, presence of urinary bilirubin and urobilin and abnormal liver function tests. Any electrolyte disturbance reflects the severity of the diarrhea and dehydration. The blood sugar may be normal or elevated

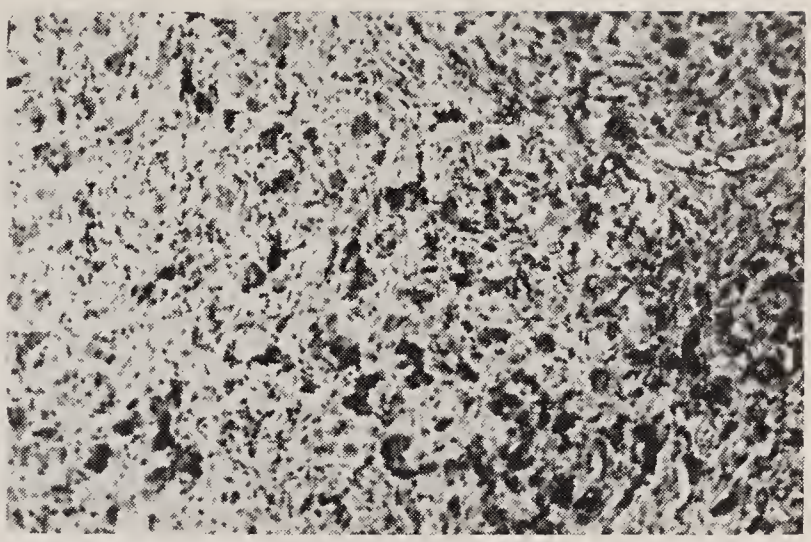

FIG. 1. Low power view of liver showing loss of normal architecture and the marked pericellular fibrosis, and parenchymal atrophy characteristic of galactosemia. (Masson Stain)

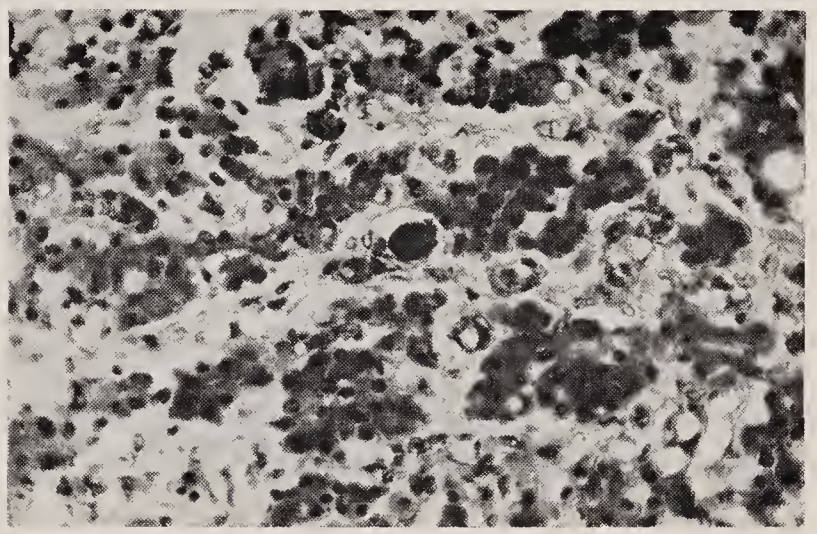

FIG. 2. High dry view of a portal area showing inspissated bile, bile duct proliferation and some evidence of fatty change in the liver cells. (Masson stain)

but the blood glucose is usually low. When the blood galactose level is high enough hvpoglycemia may be so profound as to produce convulsions. This is a result of a decreased hepatic output of glucose and an increase in insulin production. The lethargy and drowsiness so often seen are also the result of hypoglycemia. The albuminuria and amino aciduria are renal in origin as the serum amino acids remain normal. The Krebs cycle is partially blocked leading to an alteration in the proximal tubule in the reabsorption of amino acids and albumin. This occurs only in relation to galactose ingestion and galactosuria. These manifestations as well as all the laboratory and clinical findings, save the mental retardation, cirrhosis, and perhaps cataract formation, return to normal when galactose is eliminated from the diet.

Prophylaxis should be directed towards an early diagnosis. But Komrower has shown that even with early diagnosis and proper treatment galactosemics develop with a low I.Q. Psychological testing has shown their patients to be timid and fearful, and exhibiting clumsiness, poor coordination, and restlessness. These observations would seem to lend credence to the theory that the disease process actually begins in utero. It has been shown

that galactose crosses the placental barrier and the cord blood of affected infants shows an increase in Gal-1-P concentration. The logical treatment would be to limit the galactose ingestion of a known carrier of the trait during pregnancy. But this may be to no avail as lactose is manufactured during the latter weeks of pregnancy and some fetal exposure is inevitable.

There are many theories regarding the mechanisms producing the toxic manifestations seen; from a clinical standpoint, both the rate of development and rate of decline of toxicity suggest the slow accumulation of a toxic metabolite. It has long been thought that the accumulation of Gal-1-P was responsible, but the evidence for this is scant. The only definite enzymic inhibition by Gal-1-P is that of phosphoglucomutase, but the significance of this is uncertain as the glucose-6-phosphate derived from glucose is still available for normal carbohydrate metabolism. Kinoshita et al have found that dulcitol accumulates in the lens of rabbits. This is a polyalcohol derived from the reduction of galactose and not further metabolized in the lens. Thus dulcitol may be more related to cataract formation than Gal-1-P. It has been assumed for some time that the hypoglycemic episodes which occur with galactose ingestion have been casually related to the mental retardation (pathological changes in the lenticular and central nervous system). But these particular symptoms and signs of galactosemia do not occur in such clinical entities as von Gierke's disease, where episodes of hypoglycemia are profound and severe. An anemia which is partly hemolytic is present in 26% of patients according to Wang et al and could account for some of the jaundice seen. These observers believe that it is due to interference with erythrocyte O_2 consumption by Gal-1-P. Tissue damage in liver, kidney, and brain may be related to this compound by an unknown mechanism.

The incidence of galactosemia is quoted as 1/70,000 - 1/100,000 of live births but studies of Hanson et al have suggested a higher incidence of gene frequency of 1/18,000. To show manifestations of the disease in the presence of galactose, the affected individual must be homozygous for the gene concerned. Of these, 99.9% will have heterozygous parents, the remainder being due to a mutation during gestation. With new techniques, erythrocyte Gal-1-P uridyl transferase levels are determined to enable a definitive diagnosis to be made. The affected individuals show virtually no enzyme activity, while their heterozyzgous parents show approximately half the normal value. The test for this enzyme is rather sophisticated and does not lend itself easily to mass screening for heterozygotes; thus it is only after one affected offspring is born that precautions with regards to a low galactose diet in subsequent pregnancies can be instigated. Alcohol should also be avoided as it inhibits galactose utilization by the liver. Brandt et al have observed that in mongolism (Down's Syndrome) there is a 50% higher level than normal of Gal-1-P uridyl transferase. Mongolism is usually associated with a trisomy of chromosome 21, and they have concluded that the locus for control of this transferase activity is on this chromosome.

Galactosemic adults can tolerate ingested galactose much better than their infant counterparts. This may be due to the fact that the amount of ingested galactose is less with respect to body weight and surface area and/or alternate pathways develop for its improved utilization. This latter fact has been shown to occur with UDP Gal pyrophosphorylase (Enzyme V). Its activity in the adult is known to be several times greater than in the neonate. Some authors have reported further deterioration in adults who have resumed a galactose diet; therefore some still prefer their patients to continue the dietary regimen for the rest of their lives.

Many problems have been solved regarding the etiology, diagnosis and treatment of galactosemia but much is still to be learned about the pathophysiology of the disease.

As an interesting footnote to this case, the parents of this patient have subsequently had three other children and all three of them are proven galactosemics, with one dying at eight months of age. The specific enzyme test for Gal-1-P uridyl transferase was not available at the time of this patient's admission; studies on his siblings have shown no enzyme activity. His parents' erythrocyte activities are 6.2 and 6.3 units (micromoles of substrate consumed/ml erythrocytes/hr), about one-half the normal value of 20 units. The surviving siblings are 12 and 14 years of age at present and of average intelligence, showing no clinical manifestations of galactosemia. They have never been taken off the diet.

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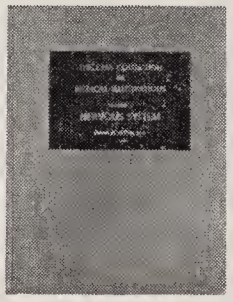

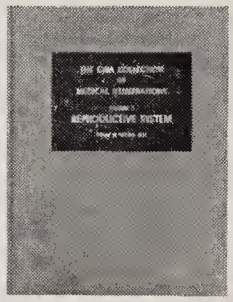

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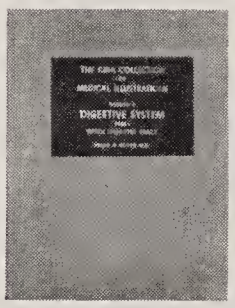

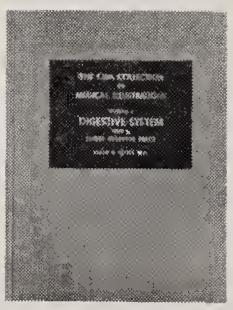

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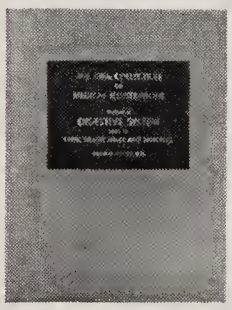

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